

## GLYCOCONJUGATES AND THEIR USE AS POTENTIAL VACCINES AGAINST INFECTION BY SHIGELLA FLEXNERI.

### FIELD OF THE INVENTION

5 This invention relates to compositions and methods for eliciting an immunogenic response in mammals, including responses that provide protection against, or reduce the severity of bacterial infections. More particularly it relates to the use of oligo- or polysaccharides obtained from natural sources and/or through synthesis or recombinant technology, and conjugates thereof to induce serum antibodies having protective activity  
10 against *Shigella flexneri*, in particular *S. flexneri* serotype 2a. These saccharides and/or conjugates thereof are useful as vaccines to induce serum antibodies which have protective activity against *S. flexneri*, in particular *S. flexneri* type 2a, and are useful to prevent and/or treat shigellosis caused by *S. flexneri*.

The present invention also relates to diagnostic tests for shigellosis using  
15 one or more of the oligo- or polysaccharides, conjugates or antibodies described above.

### BACKGROUND OF THE INVENTION

Since the discovery of *Shigella dysenteriae* type 1 (Shiga's bacillus) more than a century ago (R. Shields and W. Burnett, *Zentl. Bakterio.*, 1898, 24, 817-828), shigellosis or bacillary dysentery has been known as a serious infectious disease, occurring  
20 in humans only (T. G. Keusch and M. L. Bennish, *Shigellosis*, Plenum Medical Book Company, New York, 1991, p. 593-620). In a recent survey of the literature published between 1966 and 1997 (K. L. Kotloff, J. P. Winickoff, B. Ivanoff, J. D. Clemens, D. L. Swerdlow, P. J. Sansonetti, G. K. Adak and M. M. Levine, *Bull. WHO*, 1999, 77, 651-666), the number of episodes of shigellosis occurring annually throughout the world was  
25 estimated to be 164.7 million, of which 163.2 million were in developing countries. Up to 1.1 million annual deaths were associated with shigellosis during the same period. Occurrence of the disease is seen as a correlate of sanitary conditions, and those are not likely to improve rapidly in areas at risk.

The financial status of the populations in which shigellosis exists in its  
30 endemic forms, as well as the emerging resistance to antimicrobial drugs (M. U. Khan, *Int. J. Epidemiol.*, 1985, 14, 607-613; B. A. Iwalokun, G. O. Gbenle, S. I. Smith, A. Ogunledun, K. A. Akinsinde and E. A. Omonigbehin, *J. Health Popul. Nutr.*, 2001, 19, 183-190), limit the impact of the latter. Of the four species of *Shigellae*, *S. flexneri* is the major responsible for the endemic form of the disease, with serotype 2a being the most  
35 prevalent. The critical importance of the development of a vaccine against *Shigellae* infections was first outlined in 1987 (World Health and Organization, *Bull. W.H.O.*, 1987, 65, 17-25). Due to increasing resistance of all groups of *Shigellae* to antibiotics (S. Ashkenazi, M. May-Zahav, J. Sulkes and Z. Samra, *Antimicrob. Agents Chemother.*, 1995,

39, 819-823) vaccination remained a high priority as stated by the World Health Organization ten years later (WHO, *Weekly Epidemiol. Rec.*, 1997, 72, 73-79). In the meantime, several experimental vaccines have gone through field evaluation (T. S. Coster, C. W. Hoge, L. L. van der Verg, A. B. Hartman, E. V. Oaks, M. M. Venkatesan, D. Cohen, G. Robin, A. Fontaine-Thompson, P. J. Sansonetti and T.L. Hale, *Infect. Immun.*, 1999, 67, 3437-3443; J. H. Passwell, E. Harlev, S. Ashkenazi, C. Chu, D. Miron, R. Ramon, N. Farzan, J. Shiloach, D. A. Bryla, F. Majadly, R. Roberson, J. B. Robbins and R. Schneerson, *Infect. Immun.*, 2001, 69, 1351-1357) but there are as yet no licensed vaccines for shigellosis.

*Shigella's* lipopolysaccharide (LPS) is a major surface antigen of the bacterium. The corresponding O-SP domain (O-SP) is both an essential virulence factor and the target of the infected host's protective immune response (D. Cohen, M. S. Green, C. Block, T. Rouach and I. Ofek, *J. Infect. Dis.*, 1988, 157, 1068-1071; D. Cohen, M. S. Green, C. Block, R. Slepon and I. Ofek, *J. Clin. Microbiol.*, 1991, 29, 386-389). Indeed, using the pulmonary murine model for shigellosis, it was demonstrated that the presence locally, preliminary to infection, of a secretory antibody of isotype A specific for an epitope located on the O-SP moiety of the LPS of *S. flexneri* 5a, prevented any host homologous infection (A. Phalipon, M. Kauffmann, P. Michetti, J.-M. Cavaillon, M. Huerre, P. Sansonetti and J.-P. Krahenbuhl, *J. Exp. Med.*, 1995, 182, 769-778). Based on the former hypothesis that serum IgG anti-LPS antibodies may confer specific protection against shigellosis (J. B. Robbins, C. Chu and R. Schneerson, *Clin. Infect. Dis.*, 1992, 15, 346-361), several polysaccharide-protein conjugates, targeting either *Shigella sonnei*, *S. dysenteriae* 1 or *S. flexneri* serotype 2a, were evaluated in humans (J. H. Passwell, E. Harlev, S. Ashkenazi, C. Chu, D. Miron, R. Ramon, N. Farzan, J. Shiloach, D.A. Bryla, F. Majadly, R. Roberson, J. B. Robbins and R. Schneerson, *Infect. Immun.*, 2001, 69, 1351-1357; D. N. Taylor, A. C. Trofa, J. Sadoff, C. Chu, D. Bryla, J. Shiloach, D. Cohen, S. Ashkenazi, Y. Lerman, W. Egan, R. Schneerson and J. B. Robbins, *Infect. Immun.*, 1993, 61, 3678-3687). In the case of *S. sonnei*, recent field trials allowed Robbins and co-workers to demonstrate the efficacy of a vaccine made of the corresponding detoxified LPS covalently linked to recombinant exoprotein A (D. Cohen, S. Ashkenazi, M. S. Green, M. Gdalevich, G. Robin, R. Slepon, M. Yavzori, N. Orr, C. Block, I. Ashkenazi, J. Shemer, D. N. Taylor, T. L. Hale, J. C. Sadoff, D. Pavliovka, R. Schneerson and J. B. Robbins, *The Lancet*, 1997, 349, 155-159). Conversion of polysaccharide T-independent antigens to T-dependent ones through their covalent attachment to a carrier protein has had a tremendous impact in the field of bacterial vaccines. Several such neoglycoconjugate vaccines are currently in use against *Haemophilus influenzae* b (R. W. Ellis and D. M. Granoff, *Development and clinical use of Haemophilus b conjugate vaccines*, Dekker, New York, 1994), *Neisseria meningitidis* (P. Richmond, R. Borrow, E. Miller, S. Clark, F. Sadler, A.

Fox, N. Begg, R. Morris and K. Cartwright, *J. Infect. Dis.*, 1999, 179, 1569-1572) and *Streptococcus pneumoniae* (M. B. Renels, K. M. Edwards, H. L. Keyserling, K. Reisinger, D. A. Hogerman, D. V. Madore, I. Chang, P. R. Paradiso, F. J. Malinoski and Kimura, *Pediatrics*, 1998, 101, 604-611). These polysaccharide-protein conjugate vaccines are highly complex structures, whose immunogenicity depends on several parameters amongst which are the length and nature of the saccharide component as well as its loading on the protein. It is reasonably admitted that control of these parameters is somewhat difficult when dealing with polysaccharides purified from bacterial cell cultures. As recent progress in carbohydrate synthesis allows access to complex saccharides, it has been suggested that the use of well-defined synthetic oligosaccharides may allow a better control, and consequently the optimisation, of these parameters. Indeed, available data on *S. dysenteriae* type 1 indicate that neoglycoconjugates incorporating di-, tri- or tetramers of the O-SP repeating unit were more immunogenic than a detoxified LPS-human serum albumin conjugate of reference (V. Pozsgay, C. Chu, L. Panell, J. Wolfe, J. B. Robbins and R. Schneerson, *Proc. Natl. Acad. Sci. USA*, 1999, 96, 5164-5197).

Besides, recent reports demonstrate that short oligosaccharides comprising one repeating unit may be immunogenic in animal models (B. Benaissa-Trounev, D. J. Lefebvre, J. P. Kamerling, J. F. G. Vliegenthart, K. Kraaijeveld and H. Snippe, *Infect. Immun.*, 2001, 69, 4698-4701; F. Mawas, J. Niggemann, C. Jones, M. J. Corbet, J. P. Kamerling and J. F. G. Vliegenthart, *Infect. Immun.*, 2002, 70, 5107-5114). Another critical parameter in the design of neoglycoconjugate vaccines is the carrier protein. A potential application for these vaccines is expanding, the need for new carrier proteins licensed for human use is growing (J. B. Robbins, R. Schneerson, S. C. Szu and V. Pozsgay in *Polysaccharide-protein conjugate vaccines*, vol. 1 (S. Plotkin and B. Fantini, Eds), Elsevier, Paris, 1996, pp. 135-143). That synthetic peptides representing immunodominant T-cell epitopes could act as carriers in polysaccharide and oligosaccharide conjugates has been suggested (G. J. P. H. Boons, P. Hoogerhout, J. T. Poolman, G. A. van der Marel and J. H. van Boom, *Bioorg. Med. Chem.*, 1991, 1, 303-308) and later on demonstrated (E. Lett, S. Gangloff, M. Zimmermann, D. Wachsmann and J.-P. Klein, *Infect. Immun.*, 1994, 62, 785-792; A. Kandil, N. Chan, M. Klein and I. Chong, *Glycoconjugate J.*, 1997, 14, 13-17). Besides, the use of T-cell epitopes offers several advantages, including potential access to well-defined conjugates with no risk of epitopic suppression, as this latter phenomenon appeared to be a major drawback of protein carriers (T. Barington, M. Skettrup, L. Juul and C. Heilmann, *Infect. Immunol.*, 1993, 61, 432-438; M.-P. Schutze, C. Leclerc, M. Jolivet, F. Audibert and L. Chedid, *J. Immunol.*, 1985, 135, 2319-2322). Polypeptides containing multiple T-cell epitopes have been generated in order to address the extensive polymorphism of HLA molecules (P. R. Paradiso, K. Dermody and S. Pillai, *Vaccine Res.*, 1993, 2, 239-248). In other strategies

universal T-helper epitopes compatible with human use have been characterized, for example from tetanus toxoid (D. Valmori, A. Pessi, E. Bianchi and G. P. Corradin, *J Immunol.*, 1992, 149, 717-721), or engineered such as the pan HLA DR-binding epitope (PADRE) (J. Alexander, J. Sidney, S. Southwood, J. Ruppert, C. Oseroff, A. Maewal, K. Snoke, H. M. Serra, R. T. Kubo, A. Sette and H. M. Grey, *Immunity*, 1994, 1, 751-761). Recently, covalent attachment of the human milk oligosaccharide, lacto-N-fucopentose II to PADRE resulted in a linear glycopeptide of comparable immunogenicity to that of a glycoconjugate employing human serum albumine (HAS) as the carrier (J. Alexander, A. F. d. Guercio, A. Maewal, L. Qiao, J. Fikes, R. W. Chesnut, J. Paulson, D. R. Bundle, S. DeFrees and A. Sette, *J. Immunol.*, 2000, 164, 1625-1633).

Based on these converging data, the inventors have focused on the development of well-defined neoglycoconjugate as an alternative to polysaccharide protein conjugate vaccines targeting infections caused by *S. flexneri* serotype 2a. The target neoglycoconjugates were constructed by covalently linking an immunocarrier, serving as a T-helper epitope(s), to appropriate carbohydrate (oligo- or polysaccharide) haptens serving as B epitopes mimicking the *S. flexneri* 2a O-Ag. To this end, a rationale approach involving a preliminary study of the interaction between the bacterial O-SP and homologous protective monoclonal antibodies, was employed to define the carbohydrate haptens.

## SUMMARY OF THE INVENTION

Abbreviation: LPS: lipopolysaccharide; O-SP: O-specific polysaccharide. TT: tetanus toxoid; DCC: dicyclohexyl carbodiimide; Rhap: rhamnopyranosyl; Glcp glucopyranosyl; GlcNAcp: 2-acetamido-2-deoxy-glucopyranosyl.

In the instant invention, the list of polysaccharides designated L1 consists of:

(X)<sub>x</sub>-{B(E)C}-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{(E)CD}-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{AB(E)C}-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{B(E)CD}-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{(E)CDA}-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{DAB(E)C}<sub>n</sub>-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{B(E)CDA}<sub>n</sub>-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{(E)CDAB}<sub>n</sub>-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{AB(E)CD}<sub>n</sub>-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{DAB(E)CD}-(Y)<sub>y</sub>  
 (X)<sub>x</sub>-{B(E)CDAB(E)C}-(Y)<sub>y</sub>

wherein:

A is an alphaLRhap-(1,2) residue



B is an alphaLRhap-(1,3) residue

C is an alphaLRhap-(1,3) residue

E is an alphaDGlc-(1,4) residue

D is a betaDGlcNAc-(1,2) residue

5 x and y are independently selected among 0 and 1

X and Y are independently selected among A, B, C, D, E, AB, B(E), (E)C, CD, DA, AB(E), B(E)C, (E)CD, CDA, AB(E)C, B(E)CD, (E)CDA, CDAB, DAB(E) and wherein n is an integer comprised between 1 and 10 covalently bound to a carrier.

Saccharides selected from the group consisting of:

10 {B(E)CD}

{(E)CDAB}<sub>n</sub>

{AB(E)CD}<sub>n</sub>

wherein A,B, C, D, E and n have the same meaning as above are new and are another object of the invention.

15 It is an object of the present invention to produce an antigen based on natural, modified-natural, synthetic, semi-synthetic or recombinant oligo- or polysaccharides which have subunits, selected from the list L1. Preferably, these oligo- or polysaccharides of the invention are antigenically similar to an antigenic determinant of the O-SP of *S. flexneri* type 2a which contains [AB(E)CD] subunits. It is also an object of the invention to provide molecules, for example oligo- or polysaccharides, which are structurally related and/or antigenically similar to those oligo- and polysaccharides from the list L1. The oligo- or polysaccharides may be conjugated to an immunocarrier to form conjugates. These conjugates thereof are immunogenic and elicit serum antibodies that are protective against *S. flexneri*, in particular *S. flexneri* type 2a and which are useful in the prevention and treatment of shigellosis caused by *S. flexneri*. These oligo- or polysaccharides and conjugates thereof, and the antibodies which they elicit, are also useful for studying *S. flexneri*, in particular *S. flexneri* type 2a, *in vitro* or its products in patients. The oligo- or polysaccharides may also be conjugated to other carriers which are suitable for labelling or immobilizing said oligo- or polysaccharides on a solid phase.

30 It is yet another object of the present invention to provide an immunogen that elicits antibodies which are protective against *S. flexneri*, in particular *S. flexneri* type 2a and which react with, or bind to the O-SP of *S. flexneri* type 2a, wherein the immunogen is based on a natural, modified natural, synthetic, semi-synthetic or recombinant oligo- or polysaccharide containing one or more subunits selected from the list L1 or a structurally related, immunologically similar, oligo- or polysaccharide, and/or conjugate thereof.

35 It is yet another object of the present invention to provide antibodies which have protective activity against *S. flexneri*, in particular *S. flexneri* type 2a, and which react with, or bind to the O-SP of *S. flexneri* type 2a, wherein the antibodies may be

elicited by immunization with a natural, modified natural, or synthetic oligo- or polysaccharide containing subunits from the list L1 or a structurally related immunologically similar, oligo- or polysaccharide, and/or conjugate thereof.

5 It is yet another object of the present invention to provide oligo- or polysaccharides or conjugates thereof with a carrier which are useful as vaccines to prevent and/or treat shigellosis.

It is yet another object of the present invention to prepare antibodies for the treatment of established shigellosis. Antibodies elicited by the molecules of the invention are able to provide passive protection to an individual exposed to *S. flexneri*, in particular *S. flexneri* type 2a, to prevent, treat, or ameliorate infection and disease caused by the microorganism.

10 It is yet another object of the present invention to provide diagnostic tests and/or kits for shigellosis caused by *S. flexneri*, in particular *S. flexneri* type 2a, using one or more of the oligo- or polysaccharides, conjugates, or antibodies of the present invention

15 It is yet another object of the present invention to provide an improved method for synthesizing an oligo- or polysaccharide containing one or more subunits of the list L1.

According to the present invention, methods are provided to isolate, substantially purify and/or synthesize natural, modified-natural, synthetic, semi-synthetic or recombinant oligo- or polysaccharides containing subunits of the L1 list or structurally related, immunologically similar, oligo- or polysaccharides. Preferably, these oligo- and polysaccharides are structurally related and/or immunologically similar to an antigenic determinant of the O-SP of *S. flexneri* type 2a.

20 Methods are also provided to conjugate the natural, modified-natural, synthetic, semi-synthetic or recombinant oligo- or polysaccharide of the invention with a carrier.

## **DETAILED DESCRIPTION OF THE INVENTION**

### **Oligo- and polysaccharides:**

This invention provides a synthetic, semi-synthetic, natural, modified-natural or recombinant oligo- or polysaccharide containing subunits from the list L1.

30 Methods for synthesizing *S. flexneri* 2a di-, tri-, tetra-, penta- and octasaccharides are known from the prior art (F.Segat Dioury *et al.*, *Tetrahedron Asymmetry*, 13, 2002, 2211-2222; C.Costachel *et al.*, *J. Carbohydrate Chemistry*, 19(9) 2000, 1131-1150; L. Mulard *et al.*, *J. Carbohydrate Chemistry*, 19(7), 2000, 849-877; F.Belot *et al.*, *Tetrahedron Letters*, 43, 2002, 8215-8218; L. Mulard *et al.*, *Tetrahedron* 35 58, 2002, 2593-2604; L. Mulard *et al.*, *J. Carbohydrate Chemistry*, 19(2), 2000, 193-200).

An improved method to synthesize oligo- or polysaccharides is set forth in the examples below. Notably the synthesis of a deca-saccharide was performed by condensation of two pentasaccharide intermediates.

Definitions:

5       “oligosaccharide” as defined herein, is a carbohydrate containing from two to twenty monosaccharide units linked together, “oligosaccharide” is used herein in a liberal manner to denote the saccharides described herein; this usage differs from the standard definition that oligosaccharides may contain up to and including ten monosaccharide units (Joint Commission on Biological Nomenclature, Eur. J. Biochem. 10   1982, 126, 433-437).

      “polysaccharide” as defined herein, is a carbohydrate containing more than twenty monosaccharide subunits linked together.

      “structurally-related” oligo- or polysaccharide” as defined herein, is a modified oligo- or polysaccharide from the list L1, characterized by its ability to immunologically mimic the antigenic determinant of the O-SP of *S. flexneri*, in particular 15   *S. flexneri* type 2a. Such modified oligo- or polysaccharide can be obtained by structural alterations that render the modified polysaccharide antigenically similar to the antigenic determinant of the O-SP of *S. flexneri* 2a. Such a modified oligo- or polysaccharide can be obtained, for example, by means of a specific spacer constraining said oligosaccharide into the conformation it bears in the native O-SP. 20

      “immunoreact” means specific binding between an antigenic determinant-containing molecule and a molecule containing an antibody combining site such as a whole antibody molecule or a portion thereof.

      “antibody” refers to immunoglobulin molecules and immunologically active or functional fragments of immunoglobulin molecules comprising an antigen 25   recognition and binding site. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and active fragments of an immunoglobulin molecule, including those portions known in the art as Fab, Fab', F(ab')<sub>2</sub> and scFv, as well as chimeric antibody molecules.

30       “immunologically similar to” or “immunologically mimic” refers to the ability of an oligo- or polysaccharide of the invention to immunoreact with, or bind to, an antibody of the present invention that recognizes and binds to a native antigenic determinant on the O-SP of *S. flexneri* type 2a.

functional group refers to groups of atoms characterized by their specific 35   elemental composition and connectivity. Said functional groups confer reactivity upon the molecule that contains them. Common functional groups include: Primary amines: R-NH<sub>2</sub>; Primary Imines: -C(=NH)-R'; Azo: [Azo, -N=N-R'; Nitrile, -C≡N; Carboxylic acid, Carboxyl: -C(=O)OH], carboxylic acid and derivatives thereof like ester: -C(=O)O-R' or

activated ester; Carbonyl: [Aldehyde:  $-C(=O)H$ ; Ketone,  $-C(=O)-R'$ ], or derivative thereof as masked carbonyl such as acetal or thioacetal; Alkenes:  $-CH=CH-R'$ ; Alkynes  $-C\equiv C-R'$ ; Isocyanates:  $-N=C=O$ ; Isothiocyanate:  $-N=C=S$ ; Thioacyl  $-SCO-R'$ , Thiol SH, dithiol:  $-S-S-R'$ ; Azide  $-N_3$ ; Hydrazide:  $-CONHNH_2$ , Hydrazine, Maleimide, O-alkyl hydroxylamine, halogen,

“carrier” refers to any molecule which can be covalently bound to an oligo- or polysaccharide of the invention to form the glycoconjugate of the invention. It includes immunocarriers for use as vaccine and other carriers for preparing diagnostic reagents.

“immunocarrier” refers to an immunogenic molecule or a fragment of a molecule which is recognized by T cells and is able to induce an antibody response.

“other carriers for preparing diagnostic reagents” refers to agents commonly used to immobilize molecules onto a solid phase or to label molecules.

“a label” refers to any substance which can produce a signal which can be detected by any appropriate mean.

“glycoconjugate” refers to an oligo- or polysaccharide from the list L1 covalently bound to a carrier.

“prevention and treatment” refers to the prevention of infection or reinfection, reduction or elimination of the symptoms, and reduction or complete elimination of the pathogen. Treatment may be effected prophylactically (prior to infection) or therapeutically (following infection).

#### Oligo- and polysaccharide conjugates (glycoconjugate)

The oligo- or polysaccharides of the invention can be bound covalently to a protein or peptide carrier. This covalent bond can be a direct bond between the oligo- or polysaccharide and the peptide or protein.

According to another variant, the oligo- or polysaccharide of the list L1 can be linked to the protein or peptide via a spacer molecule. The oligo or polysaccharide can be functionalized by an  $-O-R-Z$  group, wherein R is an alkyl group comprising 1 to 12 carbon atoms, preferably 1 to 6 carbon atoms, preferably an ethyl group, and Z is a functional group which reacts with a functional group of the protein or peptide carrier. Preferably Z is  $-NH_2$ .

The oligo and polysaccharide of the list L1 bearing an  $-O$ -alkyl-Z and preferably those bearing an  $-O$ -alkyl- $NH_2$  spacer molecule are another object of the instant invention.

Notably molecules :

- {B(E)C}  $-O-R-NH_2$
- {(E)CD}  $-O-R-NH_2$
- {AB(E)C}  $-O-R-NH_2$

$\{B(E)CD\}-O-R-NH_2$   
 $\{(E)CDA\}-O-R-NH_2$   
 $\{DAB(E)C\}_n-O-R-NH_2$   
 $\{B(E)CDA\}_n-O-R-NH_2$   
 $\{(E)CDAB\}_n-O-R-NH_2$   
 $\{AB(E)CD\}_n-O-R-NH_2$   
 $\{DAB(E)CD\}-O-R-NH_2$   
 $\{B(E)CDAB(E)C\}-O-R-NH_2$

5 wherein A, B, C, D, E and n have the same meaning as above are of special interest.

10 The oligo- or polysaccharide functionalized by an -O-alkyl-NH<sub>2</sub> group, is then transformed in manner known to the man skilled in the art in an -O-alkyl-NH-CC CH<sub>2</sub>-R', wherein -R' is selected among a S-acetyl group, a linear haloalkyl group havin  
15 from 1 to 7, and preferably 1 to 3 atoms of carbone, and preferably wherein the halogen is Br, and linear carboxylic acid group having preferably 2 to 3 atoms of carbon, For example, the functionalized oligo- or polysaccharides with a S-acetyl group can be deprotected resulting in the free thiol to be reacted with a carrier which is functionalized by a haloacetyl or a maleimide group. Another strategy consists in establishing a bond  
20 between the oligo- or polysaccharide and the protein or peptide via a spacer bearing a β alanine.

According to another variant of the invention, oligo- and polysaccharide of the L1 list are terminated by an -OQ group, wherein Q is selected among alkyl and alkenyl groups comprising 1 to 12 carbon atoms. Preferably Q is selected among methyl and allyl. Particularly, a saccharide derivative selected from the group consisting of:

25  $\{B(E)CD\}-OQ$   
 $\{(E)CDAB\}_n-OQ$   
 $\{AB(E)CD\}_n-OQ$   
 $\{DAB(E)C\}_m-OQ$   
 $\{B(E)CDA\}_m-OQ$   
30  $\{DAB(E)CD\}-OQ$

wherein A, B, C, D, E and n have the same meaning as above and m is comprised from 2 and 10.

Methods for binding oligo- and/or polysaccharides to a non-toxic non-host protein are well known in the art. For example, in U.S. Patent 5,204,098 and U.S. Patent 5,738,855 it is taught that an oligo- or polysaccharide containing at least one  
35 carboxyl group, through carbodiimide condensation, may be thiolated with cystamine, or aminated with adipic dihydrazide, diaminoesters, ethylenediamine and the like. Groups which could be introduced by the method, or by other methods known in the art, include

thiols, hydrazides, amines and carboxylic acids. Both the thiolated and the aminated intermediates are stable, may be freeze dried, and stored in cold. The thiolated intermediate may be reduced and covalently linked to a polymeric carrier containing a disulfide group such as a 2-pyridyldithio group. The aminated intermediate may be covalently linked to :  
5 polymeric carrier containing a carboxyl group through carbodiimide condensation.

The oligo- or polysaccharide can be covalently bound to a carrier with or without a linking molecule. To conjugate without a linker, for example, a carboxyl-group containing oligo- or polysaccharide and an amino-group-containing carrier are mixed in the presence of a carboxyl activating agent, such as a carbodiimide, in a choice of solvent  
10 appropriate for both the oligo- or polysaccharide and the carrier, as is known in the art (Szu, S.C., A.L. Stone, J.D. Robbins, R. Schneerson, and J.B. Robbins, 1987, Vi capsula polysaccharide-protein conjugates for prevention of typhoid fever. *J. Exp. Med.*, 166:1510-1524). The oligo- or polysaccharide is preferably conjugated to a carrier using a linking molecule. A linker or crosslinking agent, as used in the present invention, is preferably :  
15 small linear molecule having a molecular weight of approximately <500 daltons and is non-pyrogenic and non-toxic in the final product form.

To conjugate with a linker or crosslinking agent, either or both of the oligo- or polysaccharide and the carrier may be covalently bound to a linker first. The linkers or crosslinking agents are homobifunctional or heterobifunctional molecules, (see  
20 references provided in Bioconjugate Techniques, G. T. Hermanson, Ed, Academic Press San Diego, 1995). e.g., adipic dihydrazide, ethylenediamine, cystamine, N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl-[N-(2-iodoacetyl)- $\beta$ -alanyl propionate-propionate (SIAP), succinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (SMCC), 3,3'-dithiodipropionic acid, and the like. Among the class of  
25 heterobifunctional linkers are omega-hydroxy alkanoic acids.

According to the type of bonding between the oligo- or polysaccharide and the carrier, there is the possibility of preparing a conjugate molecule wherein the ratio of the oligo- or polysaccharide versus the carrier can vary between 1:1 and 30:1. Preferably, this ratio is comprised between 5:1 and 20:1.

30 A carrier can be a natural, modified-natural, synthetic, semi-synthetic or recombinant material containing one or more functional groups, for example primary and/or secondary amino groups, azido groups, or carboxyl group. The carrier can be water soluble or insoluble. Carriers that fulfil these criteria are well-known to those of ordinary skill in the art.

35 Immunocarriers are chosen to increase the immunogenicity of the oligo or polysaccharide and/or to raise antibodies against the carrier which are medically beneficial.

Suitable immunocarriers according to the present invention include proteins, peptides, polysaccharides, polylactic acids, polyglycolic acids, lipid aggregates (such as oil droplets or liposomes), and inactivated virus particles.

According to an advantageous embodiment of the glycoconjugate molecule of the invention, it is covalently bound to a protein or a peptide comprising at least one T-helper cell epitope, for use as a vaccine against *S. flexneri* infection.

Protein carriers known to have potent T-cell epitopes, include but are not limited to bacterial toxoids such as tetanus, diphtheria and cholera toxoids, *Staphylococcus* exotoxin or toxoid, *Pseudomonas aeruginosa* Exotoxin A and recombinantly produced genetically detoxified variants thereof, outer membrane proteins (OMPs) of *Neisseria meningitidis* and *Shigella flexneri* proteins. The recombinantly-produced, non-toxic mutant strains of *Pseudomonas aeruginosa* Exotoxin A (rEPA) are described in Fattom et al., Inf. Immun., 1993, 61, 1023-1032. The CMR 197 carrier is a well characterized non-toxic diphtheria toxin mutant that is useful in glycoconjugate vaccine preparations intended for human use (Bixler et al., Adv. Exp. Med. Biol., 1989, 251, 175-; Constantino et al. Vaccine, 1992). Other exemplary protein carriers include the Fragment C of tetanus toxin and the Class 1 or Class 2/3 OMPs. Also CRM 9 carrier has been disclosed for human immunisation. (Passwell JH et al. Pediatr Infect Dis J. (2003) 22, 701-6).

Synthetic peptides representing immunodominant T-cell epitopes can also act as carriers in polysaccharide and oligosaccharide conjugates. The peptide carriers include polypeptides containing multiple T-cell epitopes addressing the extensive polymorphism of HLA molecules (Paradiso et al., Vaccine Res., 1993, 2, 239-248), and universal T-helper epitopes compatible with human use. Exemplary T-helper epitopes include but are not limited to natural epitopes characterized from tetanus toxoid (Valmor et al., J. Immunol., 1992, 149, 717-721) and non-natural epitopes or engineered epitopes such as the pan HLA DR-binding epitope PADRE (KXVAAWTLKAA; Immunity, 1994 1, 751-761).

Other types of carrier include but are not limited to biotin. The oligo- or polysaccharides conjugated to biotin or to a label are especially designed for diagnosing *S. flexneri* infections.

#### Vaccine

The invention provides an immunogenic composition comprising a glycoconjugate as defined above, in a physiologically acceptable vehicle.

The vaccine composition includes one or more pharmaceutically acceptable excipients or vehicles such as water, saline, glycerol, ethanol. Additionally auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

The glycoconjugate of the present invention which induces protective antibodies against *S. flexneri* infection, in particular *S. flexneri* type 2a are administered to a mammal subject, preferably a human, in an amount sufficient to prevent or attenuate the severity, extent of duration of the infection by *S. flexneri*, in particular *S. flexneri* type 2a.

5 Each vaccine dose comprises a therapeutically effective amount of oligo- or polysaccharide conjugate. Such amount will vary depending on the subject being treated, the age and general condition of the subject being treated, the capacity of the subject's immune response to synthesize antibodies, the degree of protection desired, the severity of the condition to be treated, the particular oligo- or polysaccharide conjugate  
10 selected and its mode of administration, among other factors. An appropriate effective amount can be readily determined by one of skill in the art. A therapeutically effective amount will fall in a relatively broad range that can be determined through routine trials.

More particularly the oligo- or polysaccharide conjugate of the invention will be administered in a therapeutically effective amount that comprises from 1 to 1000  
15  $\mu$ g of oligo- or polysaccharide, preferably 1 to 50  $\mu$ g.

An optimal amount for a particular vaccine can be ascertained by standard studies involving measuring the anti-LPS 2a antibody titers in subjects.

Following an initial vaccination, subjects may receive one or two booster injections at about four week intervals.

20 According to a preferred embodiment of said immunogenic composition said glycoconjugates comprises a pentasaccharide or a multimer thereof such as a decasaccharide or a pentadecasaccharide

The immunogenic composition of the invention may be administered with or without adjuvant. Adjuvants can be added directly to the vaccine compositions or  
25 can be administered separately, either concurrently with or shortly after, administration of the vaccine. Such adjuvants include but are not limited to aluminium salts (aluminium hydroxide), oil-in-water emulsion formulations with or without specific stimulating agents such as muramyl peptides, saponin adjuvants, cytokines, detoxified mutants of bacterial toxins such as the cholera toxin, the pertussis toxin, or the *E.coli* heatlabile toxin.

30 The immunogenic composition of the invention may be administered with other immunogens or immunoregulatory agents, for example, immunoglobulins, cytokines, lymphokines and chemokines.

According to another preferred embodiment of said immunogenic composition, it comprises at least an immunogen which afford protection against another  
35 pathogen, such as for example, *S. flexneri* serotype 1b, 3a and 6, *S. species* such as *S. dysenteriae* 1 and *S. sonnei* or pathogens responsible for diarrhoeal disease in humans [*Vibrio cholerae* (cholera), *Salmonella typhimurium* (typhoid), rotavirus, Enterotoxigenic strains of *E. Coli* (ETEC)].



Typically, the vaccine compositions are prepared as injectables either as liquid solutions or suspensions; or as solid forms suitable for solution or suspension in liquid vehicle prior to injection. The preparation may be emulsified or encapsulated in liposomes for enhanced adjuvant effect.

5                   Once formulated, the vaccine compositions may be administered parenterally, by injection, either subcutaneous, intramuscular or intradermal.

Alternative formulations suitable for other mode of administration include oral and intranasal formulations.

#### Antibodies

10                   The invention provides monoclonal IgG antibodies immunoreactive with a serotype 2a-specific antigenic determinant of the O-SP of *S. flexneri* type 2a (O-SP or O-Ag) which are produced by an hybridoma cell line deposited under the accession number I-3197, I-3198, I-3199, I-3200 and I-3201, on april, 20, 2004, at the Collection Nationale de Cultures de Microorganismes, INSTITUT PASTEUR, 25 rue du Docteur Roux, 75724  
15   PARIS CEDEX 15, FRANCE.

The invention encompasses also the hybridoma cell line producing the here above defined monoclonal IgG antibodies.

The monoclonal IgG antibodies according to the invention are representative of the different IgG subclasses;

20                   - the hybridoma cell line I-3197 produces an IgG2a antibody denominated hereafter A2-1,

- the hybridoma cell line I-3198 produces an IgG3 antibody denominated hereafter C1-7,

25                   - the hybridoma cell line I-3199 produces an IgG1 antibody denominated hereafter D15-7,

- the hybridoma cell line I-3200 produces an IgG2b antibody denominated hereafter E4-1,

the hybridoma cell-line I-3201 produces an IgG1 antibody denominated hereafter F22-4.

30                   The invention provides also chimeric antibodies comprising : (i) a fragment of the heavy and/or light chain(s) which is identical with or homologous to the sequences of one of the here above defined mouse monoclonal IgG antibody, and (ii) the remainder of the heavy and or light chain(s) which is identical with or homologous to the sequences of an antibody from another species or belonging to another antibody class or  
35   subclass.

Accordingly, an advantageous embodiment of said chimeric antibody, is a humanized antibody which contains minimal sequences from mouse origin. For the most part humanized antibodies are human immunoglobulins in which the residues from one or

more CDR(s) are replaced by residues from one or more CDR(s) of one of the here above defined mouse monoclonal IgG antibodies. Furthermore, humanized antibody may comprise residues which are found neither in the human antibody, nor in the imported CDR(s) or framework (FR) sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains in which all or substantially all of the CDR regions correspond to those of the mouse monoclonal IgG antibody as here above defined, and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a domain of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

Preferably, said humanized antibody comprises the constant region from an IgG or an IgA, or at least the CH3 domains thereof.

More preferably, when said constant region is from an IgA, said humanized antibody comprises also a J chain so as to form dimeric IgA and/or a secretory component, so as to form secretory IgA.

According to another advantageous embodiment of said chimeric antibody it comprises a Fab fragment from said mouse monoclonal IgG antibody and a constant region from a human IgA, or at least the CH3 domains thereof.

The invention provides also fragments from the here above defined monoclonal IgG antibodies and deriving chimeric antibodies. Preferred fragments are functional fragments comprising the antigen recognition and binding site such as: Fv or half of the Fv comprising only three Complementarity-Determining-Regions (CDRs), Fab and Fab'<sub>2</sub>.

Accordingly, an advantageous embodiment of said fragments is the CDR defined by the sequences SEQ ID NO: 12 to 34.

The invention provides also the polynucleotides (DNA or RNA) encoding the heavy and/or light chain from the here above defined antibodies, or a fragment thereof such as: a variable region (VL, VH) or a portion thereof such as a framework and/or CDR, and a constant region or a portion thereof such as a constant domain (CL, CH1, CH2, CH3).

The invention provides also the vectors comprising said polynucleotides.

The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of preferred vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors A vector according to

the present invention comprises, but is not limited to, a YAC (yeast artificial chromosome), a BAC (bacterial artificial), a baculovirus vector, a phage, a phagemid, a cosmid, a viral vector, a plasmid, a RNA vector or a linear or circular DNA or RNA molecule which may consist of chromosomal, non chromosomal, semi-synthetic or synthetic DNA. In general, expression vectors of utility in recombinant DNA techniques are often in the form of “plasmids” which refer generally to circular double stranded DNA loops which, in their vector form are not bound to the chromosome. Large numbers of suitable vectors are known to those of skill in the art.

Preferably said vectors are expression vectors, wherein a sequence encoding an antibody of the invention is placed under control of appropriate transcriptional and translational control elements to permit production or synthesis of said protein. Therefore, said polynucleotide is comprised in an expression cassette. More particularly, the vector comprises a replication origin, a promoter operatively linked to said encoding polynucleotide, a ribosome site, an RNA-splicing site (when genomic DNA is used), a polyadenylation site and a transcription termination site. It also can comprise an enhancer. Selection of the promoter will depend upon the cell in which the polypeptide is expressed.

The invention also concerns a prokaryotic or eukaryotic host cell that is modified by a polynucleotide or a vector as defined above, preferably an expression vector.

As used herein, a cell refers to a prokaryotic cell, such as a bacterial cell, or eukaryotic cell, such as an animal, plant or yeast cell.

The invention also concerns a non-human transgenic animal or a transgenic plant, wherein all or part of the cells are modified by a polynucleotide or a vector as defined above.

The polynucleotide sequence encoding the polypeptide of the invention may be prepared by any method known by the man skilled in the art. For example, it is amplified from a cDNA template, by polymerase chain reaction with specific primers. Preferably the codons of said cDNA are chosen to favour the expression of said protein in the desired expression system.

The recombinant vectors comprising said polynucleotide may be obtained and introduced in a host cell by the well-known recombinant DNA and genetic engineering techniques.

The antibody of the invention may be obtained by culturing the host cell containing an expression vector comprising a polynucleotide sequence encoding said polypeptide, under conditions suitable for the expression of the polypeptide, and recovering the polypeptide from the host cell culture.

#### Passive protection

The invention provides a pharmaceutical composition comprising an antibody, as defined above or a functional fragment thereof, and a physiologically acceptable vehicle.

5 The antibodies of the present invention which have a protective effect against *S. flexneri* infection, in particular *S. flexneri* type 2a are administered to a mammal subject, preferably a human, in an amount sufficient to prevent or attenuate the severity, extent of duration of the infection by *S. flexneri*, in particular *S. flexneri* type 2a.

10 The administration of the antibody may be either prophylactic (prior to the anticipated exposure to *S. flexneri*) or therapeutical (after the initiation of the infection, at or shortly after the onset of the symptoms).

The dosage of the antibodies will vary depending upon factors as the subject's age, weight and species. In general, the dosage of the antibody is in the range of from about 1 mg/kg to 10 mg/kg body weight.

15 Preferably, said antibody is a humanized antibody of the IgG or the IgA class.

The route of administration of the antibody may be oral or systemic, for example, subcutaneous, intramuscular or intravenous.

#### Diagnosis

20 The antibodies and the oligo- or polysaccharides according to the present invention are used, *in vitro*, as *S. flexneri* type 2a specific diagnostic reagents in standard immunoassays.

The antibodies according to the present invention are used to test for the presence of *S. flexneri* type 2a in biological samples, for establishing the diagnosis of shigellosis in an individual presenting a diarrhoeal disease.

25 Alternatively, the oligo- or polysaccharides according to the present invention are used to test the presence of *S. flexneri* type 2a-specific antibodies. Oligo- or polysaccharides may be used for epidemiological studies, for example for determining the geographic distribution and/or the evolution of *S. flexneri* type 2a infection worldwide, as well as for evaluating the *S. flexneri* type 2a-specific antibody response induced by an immunogen.

30 The antibodies and the oligo- or polysaccharides according to the present invention may be advantageously labelled and/or immobilized onto a solid phase, according to standard protocols known to the man skilled in the art. Such labels include, but are not limited to, enzymes (alkaline phosphatase, peroxydase), luminescent or fluorescent molecules. For example an oligo- or polysaccharide conjugated to biotine,

according to the present invention may be immobilized onto a solid phase, to detect the presence of *S. flexneri* type 2a-specific antibodies in biological samples.

Such immunoassays include, but are not limited to, agglutination assays, radioimmunoassay, enzyme-linked immunosorbent assays, fluorescence assays, western-blots and the like.

Such assays may be for example, of direct format (where the labelled antibody /oligo- or polysaccharide is reactive with the antigen/antibody to be detected), an indirect format (where a labelled secondary antibody is reactive with said antibody/oligo- or polysaccharide), a competitive format (addition of a labelled antibody/oligo- or polysaccharide), or a sandwich format (where both labelled and unlabelled antibodies are used).

For all therapeutic, prophylactic and diagnostic uses, the oligo- or polysaccharides of the invention, alone or linked to a carrier, as well as antibodies and other necessary reagents and appropriate devices and accessories may be provided in kit form so as to be readily available and easily used.

#### Detailed description of the preparation of the molecules

The instant invention is based on the characterization of the antigenic determinants of *S. flexneri* 2a O-SP recognized by serotype-specific protective monoclonal antibodies. The synthesis, as their methyl glycosides, of a panel of oligosaccharides representative of fragments of *S. flexneri* 2a O-SP was thus undertaken to be used as probes in the study of antibody recognition.

A	B	E	C	D
2)- $\alpha$ -L-Rhap-(1 $\rightarrow$ 2)- $\alpha$ -L-Rhap-(1 $\rightarrow$ 3)-[ $\alpha$ -D-Glcp-(1 $\rightarrow$ 4)]- $\alpha$ -L-Rhap-(1 $\rightarrow$ 3)- $\beta$ -D-				
25	GlcNAcp(1 $\rightarrow$			

The O-SP of *S. flexneri* 2a is a heteropolysaccharide defined by the pentasaccharide repeating unit I. {(D. A. R. Simmons, *Bacteriol. Reviews* **1971**, *35*, 117-148; A. A. Lindberg, A. Kamell, A. Weintraub, *Rev. Infect. Dis.* **1991**, *13*, S279-S284) It features a linear tetrasaccharide backbone, which is common to all *S. flexneri* O-antigens, except serotype 6, and comprises a *N*-acetyl glucosamine and three rhamnose residues, together with an  $\alpha$ -D-glucopyranose residue branched at position 4 of rhamnose C. We have already reported on the synthesis of the methyl glycosides of various fragments of the O-SP, including the known EC disaccharide, (J. M. Berry, G. G. S. Dutton, *Can. J. Chem.* **1974**, *54*, 681-683; G. M. Lipkind, A. S. Shashkov, A. V. Nikolaev, S. S. Mamyan, N. K. Kochetkov, *Bioorg. Khim.* **1987**, *13*, 1081-1092; L. A. Mulard, C. Costachel, P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, *19*, 849-877) the ECD (L. A. Mulard, C. Costachel,

P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, *19*, 849-877) and **B(E)C**(L. A. Mulard, C. Costachel, P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, *19*, 849-877) trisaccharides, the **ECDA**(F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) and **AB(E)C**(C. Costachel, P. J. Sansonetti, L. A. Mulard, *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150) tetrasaccharides, the **B(E)CDA**(F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) and **DAB(E)C**(C. Costachel, P. J. Sansonetti, L. A. Mulard, *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150) pentasaccharides and more recently the **B(E)CDAB(E)C** octasaccharide(F. Bélot, C. Costachel, K. Wright, A. Phalipon, L. A. Mulard, *Tetrahedron. Lett.* **2002**, *43*, 8215-8218).

10 In the following, we report on the synthesis of the **ECDAB**, **AB(E)CD** pentasaccharides as well on that of the **B(E)CD** tetrasaccharide as their methyl glycosides, **1**, **2** and **3**, respectively. We also report on the synthesis of a pentasaccharide **DAB(E)C** building block (**201**) and that of the corresponding trichloroacetimidate donor **203**. The decasaccharide **D'A'B'(E')C'DAB(E)C** fragment, was prepared as its methyl glycoside

15 (**301**).

**I- Synthesis of oligo- and polysaccharides according to the invention**

**A- Synthesis of a tetra- and two pentasaccharide fragments of the O-specific polysaccharide of *Shigella flexneri* serotype 2a:**

The synthesis of the methyl glycosides of the **ECDAB**, **AB(E)CD** pentasaccharides and that of the **B(E)CD** tetrasaccharide, **101**, **102** and **103**, respectively, is reported in the following.

Analysis of the targets shows that all the glycosylation reactions to set up involve 1,2-*trans* glycosidic linkages except for that at the **E-C** junction which is 1,2-*cis*. Consequently, the syntheses described herein rely on key **EC** disaccharide building blocks as well as on appropriate **A**, **B** and **D** monosaccharide synthons.

*Synthesis of the linear ECDAB-OMe pentasaccharide (101):* Based on earlier findings in the series which have demonstrated that the **C-D** linkage was an appropriate disconnection site. (F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222; F. Bélot, C. Costachel, K. Wright, A. Phalipon, L. A. Mulard, *Tetrahedron. Lett.* **2002**, *43*, 8215-8218; F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074) Consequently, the synthesis of **101** was designed (Figure 1) based on the glycosylation of the known **EC** trichloroacetimidate donor **114**, (L. A. Mulard, C. Costachel, P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, *19*, 849-877) obtained in three steps (69%) from the key diol **113**, (F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) and the **DAB** trisaccharide acceptor **112**. The latter was obtained by the stepwise condensation of known monosaccharide precursors, readily available by selective protection, deprotection and activation sequences. Thus, TMSOTf-catalysed condensation of the rhamnopyranoside acceptor **104** (V. Pozsgay, J.-R. Brisson,

H. J. Jennings, *Can. J. Chem.* **1987**, *65*, 2764-2769) with the trichloroacetimidate donor 5(J. C. Castro-Palomino, M. H. Rensoli, V. V. Bencomo, *J. Carbohydr. Chem.* **1996**, *15*, 137-146) in diethyl ether to give the fully protected rhamnobioside **106**, and subsequent de-*O*-acetylation gave the **AB** disaccharide acceptor **107** in 91% overall yield, which  
5 compares favourably with the previously described preparation using the corresponding 1-*O*-acetyl donor.(V. Pozsgay, J.-R. Brisson, H. J. Jennings, *Can. J. Chem.* **1987**, *65*, 2764-2769) Analogously to previous work in a related series,(F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074) the known glucosaminyl trichloroacetimidate donor **109**,(J. C. Castro-Palomino, R. R. Schmidt, *Tetrahedron Lett.*  
10 **1995**, *36*, 5343-5346) was chosen as the precursor to residue **D**. Conventional glycosylation of **107** with **109** was best performed in acetonitrile using tin trifluoromethanesulfonate ( $\text{Sn}(\text{OTf})_2$ ) as the catalyst(A. Lubineau, A. Malleron, *Tetrahedron Lett.* **1985**, *26*, 1713-1716) to give the fully protected trisaccharide **110** in 72% yield (extracted from the  $^1\text{H}$  NMR spectrum). When TMSOTf was used instead of  
15  $\text{Sn}(\text{OTf})_2$ , **110** was formed in lower yield (52%) outlining the sensitivity of the tetrachlorophthaloyl group to these stronger conditions, as previously noted.(L. Lay, L. Manzoni, R. R. Schmidt, *Carbohydr. Res.* **1998**, *310*, 157-171) A three step process including heating **110** with ethylenediamine in dry ethanol,(J. S. Debenham, R. Madsen, C. Roberts, B. Fraser-Reid, *J. Am. Chem. Soc.* **1995**, *117*, 3302-3303) ensuing *N*-acetylation  
20 with acetic anhydride, and de-*O*-acetylation under Zemplén conditions, furnished the triol **111** (51% from **107**). It was next protected at positions 4<sub>D</sub> and 6<sub>D</sub> by regioselective introduction of an isopropylidene acetal upon reaction with 2,2-dimethoxypropane under acid-catalysis to give **112** (96%). The latter acetal-protecting group was selected based on data previously obtained when synthesizing shorter fragments in the series which had  
25 outlined the interest of using 4,6-*O*-isopropylidene-glucosaminyl intermediates instead of the more common benzylidene analogues.(L. A. Mulard, C. Costachel, P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, *19*, 849-877) Once the two key building blocks were made available, their condensation was performed in dichloromethane in the presence of a catalytic amount of TMSOTf to give the fully protected pentasaccharide **115** (84%).  
30 Conventional stepwise deprotection involving (i) acidic hydrolysis of the isopropylidene acetal using 90% aq TFA to give diol **116** (95%), (ii) conversion of the latter into the corresponding tetraol **117** under Zemplén conditions (86%), and (iii) final hydrogenolysis of the benzyl protecting groups, gave the linear pentasaccharide target **101** in 81% yield.

*Synthesis of the AB(E)CD pentasaccharide 102 and of the B(E)CD tetrasaccharide 103.* For reasons mentioned above, the glucosaminyl acceptor **118**,(L. A. Mulard, C. Costachel, P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, *19*, 849-877) protected at its 4 and 6 hydroxyl groups by an isopropylidene acetal was the precursor of choice for  
35 residue **D** (Figure 2). In the past, introduction of residue **B** at position 3<sub>C</sub> was performed on

a 2<sub>C</sub>-*O*-benzoylated EC acceptor resulting from the regioselective acidic hydrolysis of the corresponding 2,3-orthoester intermediate.(F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222; C. Costachel, P. J. Sansonetti, L. A. Mulard, *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150) It rapidly occurred to us that opening of the  
5 intermediate phenyl orthoester was not compatible with the presence of 4<sub>D</sub>,6<sub>D</sub>-*O*-isopropylidene acetal. For that reason, the trichloroacetimidate donor **119**, suitably benzoylated at position 2<sub>C</sub> and orthogonally protected by a chloroacetyl group at position 3<sub>C</sub> was used as the EC building block instead of the previously used **114**. Protection at the 2-OH of the rhamnosyl precursor to residue **B** was also crucial in the synthesis of **102**.  
10 Indeed, most of our previous work in the series relied on the use of the known 2-*O*-acetyl rhamnopyranosyl donor **105**, In the reported syntheses,(C. Costachel, P. J. Sansonetti, L. A. Mulard, *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150) selective de-*O*-acetylation at position 2<sub>B</sub> in the presence of a 2<sub>C</sub>-*O*-benzoate was best performed by treatment with methanolic HBF<sub>4</sub>.OEt<sub>2</sub> for five days. Clearly, such de-*O*-acetylation conditions are not  
15 compatible with the presence of an isopropylidene acetal on the molecule. To overcome this limitation, the corresponding 2-*O*-chloroacetyl rhamnopyranosyl trichloroacetimidate **120** was selected as an alternate donor. In theory, the latter could also serve as an appropriate precursor to residue A.

Regioselective conversion of diol **113** into its 2-*O*-benzoylated  
20 counterpart **121** was performed as described (Figure 3).(F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) Treatment of the latter with chloroacetic anhydride and pyridine gave the orthogonally protected **122** (95%), which was smoothly de-*O*-allylated to yield the corresponding hemiacetal **123** (91%) by a two-step process, involving (i) iridium (I)-promoted isomerisation(J. J. Oltvoort, C. A. A. van Boeckel, J. H.  
25 der Koning, J. van Boom, *Synthesis* **1981** 305-308) of the allyl glycoside and (ii) subsequent hydrolysis in the presence of iodine.(M. A. Nashed, L. Anderson, *J. Chem. Soc. Chem. Commun.* **1982** 1274-1282) The selected trichloroacetimidate leaving group was successfully introduced by treatment of **123** with trichloroacetonitrile in the presence of DBU, which resulted in the formation of **119** (84%) together with the recovery of some  
30 starting hemiacetal (14%) since partial hydrolysis during column chromatography could not be avoided. TMSOTf-mediated glycosylation of donor **119** and acceptor **118** furnished the fully protected ECD trisaccharide (**124**, 80%), which was readily converted to the required acceptor **125** upon selective deblocking of the chloroacetyl protecting group with thiourea (97%). Following the two-step protocol described above for the preparation of  
35 **119**, the known allyl rhamnopyranoside **127**,(P. Westerduin, P. E. der Haan, M. J. Dees, J. H. van Boom, *Carbohydr. Res.* **1988**, *180*, 195-205) bearing a 2-*O*-chloroacetyl protecting group, was converted to the hemiacetal **128** (85%) (Figure 4). Next, treatment of the latter with trichloroacetonitrile and a slight amount of DBU gave at best donor **120** in a yield of



73%. Although the isolated yield of **120** was not better (72%), running the activation step in the presence of  $K_2CO_3$  instead of DBU resulted in a more reproducible isolated yield of the activated donor. Glycosylation of the ECD acceptor **125** and the B donor **120** was attempted under various conditions of solvent and catalyst. Whatever the conditions, hardly separable mixtures of compounds were obtained, among which the yield of the target tetrasaccharide reached 45-50%. Running the condensation in  $Et_2O$  in the presence of TMSOTf as the promoter were the best conditions tested, although the expected tetrasaccharide **129** was often slightly contaminated with glycosylation intermediates such as the silylated **126** or the orthoester **135** (Figure 5), as suggested from mass spectroscopy analysis and NMR data. In fact, the nature of the latter was fully ascertained at the next step in the synthesis. Indeed, full recovery of the starting material was observed upon treatment of **135** with thiourea. On the contrary, treatment of a mixture of the condensation products **129** and supposedly **126** under the same conditions led to the expected tetrasaccharide acceptor **131** and the trisaccharide acceptor **125** (not described). The  $\beta$ B-tetrasaccharide isomer could not be detected at this stage, indicating that the corresponding chloroacetylated  $\beta$ B-anomer was probably not part of the initial mixture. Formation of the starting **125** during the dechloroacetylation step was not unexpected, since loss of a trimethylsilyl group under similar treatment was observed for a model compound (not described). Although the fluoride analog corresponding to donor **120** has been used successfully in a prior report, (P. Westerduin, P. E. der Haan, M. J. Dees, J. H. van Boom, *Carbohydr. Res.* **1988**, *180*, 195-205) the poor yield of **129** may be, in part, associated to the sensitivity of the chloroacetyl group to the glycosylation conditions. Thus, in order to investigate the poor outcome of the condensation reaction, the donor properties of the chloroacetylated **120** were compared to that of the more common acetylated **105**. When methyl rhamnopyranoside **104** was condensed with **120** as described for the preparation of **106**, the rhamnobioside **108** was isolated in 67% yield. This result tends to suggest that indeed the acetylated **5** is a more powerful donor than **120**.

Starting from **120** and **125**, the isolated yield of the tetrasaccharide acceptor **131** was 34%, which encouraged us to reconsider the use of **105** as a precursor to residues B and A in the synthesis of **102**. Condensation of **105** and **125** in  $CH_2Cl_2$  using TMSOTf as the promoter furnished the corresponding tetrasaccharide **130** (72%). However, even though the yield of **131** was better than that of **129**, slight contamination by the silylated side-product **126** was again apparent, outlining the somewhat poor reactivity of the ECD acceptor. Subsequent treatment of **130** with a 0.4 M ethanolic solution of guanidine (N. Kunesh, C. Miet, J. Poisson, *Tetrahedron Lett.* **1987**, *28*, 3569-3572) resulted in selective 2<sub>B</sub>-O-deacetylation to give **131** in a satisfactory 83% yield, which outlined the interest of the method. However, previous experience in other closely related series has shown that the selectivity of the method was highly dependent on the nature of the

substrate. Clearly, the 2-*O*-acetylated donor **105** was preferred to the chloroacetate analogue **120**. Condensation of the tetrasaccharide acceptor **131** and donor **105** in the presence of TMSOTf gave the fully protected pentasaccharide **132** in a yield of 52%. TF-mediated hydrolysis of the isopropylidene acetal followed by transesterification of the ester groups and subsequent conventional hydrogenolysis of the benzyl ethers finally gave the target pentasaccharide **2** (88%).

Alternatively, the fully protected tetrasaccharide **130** was converted to the diol **136** by acidic removal of the isopropylidene acetal (85%), and subsequently to the corresponding tetraol **137** upon transesterification (83%). Final hydrogenolysis of the benzyl groups furnished the target tetrasaccharide **103** (71%) (Figure 6).

Noteworthy, in the case of intermediates **133** and **136**, removal of the esters required heating of the reaction mixtures, whereas de-*O*-acylation of **117** proceeded smoothly at rt. Occurring most probably as a consequence to the branched nature of compounds **133** and **136**, steric hindrance and isolation of the acyl groups (Z. Szirmai, A. Liptak, G. Snatzke, *Carbohydr. Res.* **1990**, *200*, 201-208) may best explain this phenomenon. Steric hindrance may also account for the poor outcome of the condensation of the ECD acceptor **125** with the B donors **120** and **105**. Interestingly, <sup>13</sup>C NMR data support this hypothesis. Although no altered signals could be seen in the <sup>13</sup>C NMR spectrum of the ECD acceptor **125** or in the <sup>13</sup>C NMR spectra of the fully protected precursor **124**, significant disturbance of several signals in the <sup>13</sup>C NMR spectra of the tetra- and pentasaccharides were seen repeatedly. At the protected and partially protected stage, major altered signals are those tentatively assigned to C-3<sub>C</sub> and C-4<sub>C</sub>. Besides, signals assigned to C-2<sub>D</sub>, C-3<sub>D</sub> as well as to C-1<sub>B</sub> are significantly broader than expected. Loss of conformational flexibility at the C ring is not totally unexpected especially since the carbons involved are those corresponding to the branching points. Of particular interest however, was the observation that residue D, the *N*-acetyl-glucosaminyl residue, was also partially constrained. Full conformational freedom of residue D is recovered when the B(E)CD and AB(E)CD oligosaccharides are in their free form. However, this observation does not stand true for residue C since characteristic broad signals for C-3<sub>C</sub> and C-4<sub>C</sub> as well for C-1<sub>B</sub> and C-1<sub>E</sub> are still present in the <sup>13</sup>C NMR spectra of compounds **102** and **103**, respectively. Overall, these observations suggest a somewhat compact organisation at the branching point of the B(E)CD structure. It is worth mentioning that none of these disturbed signals are seen in the <sup>13</sup>C NMR spectra of the oligosaccharides corresponding to the linear ECDAB fragment.

The synthesis of the methyl glycoside (**102**) of the repeating unit I of the *S. flexneri* 2a O-SP, together with that of the corresponding frame-shifted pentasaccharide **101** and tetrasaccharide **103** were described. All the methyl glycosides of the di- to pentasaccharides obtained by circular permutation of the monosaccharide residues

partaking in the linear backbone of **I**, and comprising the **EC** portion, are now available in the laboratory. Their binding to a set of protective monoclonal IgG antibodies will be reported elsewhere.

**B- Synthesis of a pentasaccharide building block of the O-specific polysaccharide of *Shigella flexneri* serotype 2a: DAB(E)C**

In the following, a synthesis of the **DAB(E)C** pentasaccharide **201**, which is protected in an orthogonal fashion at position O-3<sub>D</sub> with an acetyl group and at the reducing end by an allyl group. At this stage, the acetamido function is already present at position 2<sub>D</sub>. Compound **201** may be converted to the corresponding alcohol **202**, which acts as an donor and a masked donor, or to the trichloroacetimidate **203** which acts as an acceptor allowing subsequent chain elongation at the non-reducing end (Figure 7). Previous work in the laboratory has shown that in order to construct the **DAB(E)C** sequence, the linear approach involving stepwise elongation at the non-reducing end, was more suitable than the blockwise one.

*D-glucosamine unit(D)*. In order to limit the number of steps at the pentasaccharide level, we reasoned that an appropriate precursor to residue **D** should have (i) permanent protecting groups at positions 4 and 6, (ii) a participating group at position 2 and (iii) an orthogonal protecting group at position 3, allowing easy cleavage. As they allow a wide range of protecting group manipulations previously to ultimate activation, thioglycosides are highly convenient masked donors. Recently, two sets of non-malodorous thioglycosyl donors have been proposed (H. Dohi, Y. Nishida, T. Takeda, K. Kobayashi, *Carbohydr. Res.* **2002**, 337, 983-989; H. Matsui, J.-I. Furukawa, T. Awano, N. Nishi, N. Sakairi, *Chem. Lett.* **2000**, 29, 326-327), among which the thiododecanyl moiety was selected (Figure 8). Thus, the known peracetylated trichloroacetamide **204** (G. Blatter, J.-M. Beau, J.-C. Jacquinet, *Carbohydr. Res.* **1994**, 260, 189-202) was reacted with dodecanthiol in the presence of BF<sub>3</sub>.OEt<sub>2</sub> to give thioglycoside **205** in high yield (97%). Zemplén deacetylation cleanly afforded the corresponding triol **206**, which was selectively protected at position 4 and 6 upon reaction with 2,2-dimethoxypropane to give **207** (80% from **204**). Indeed, previous observations in the series have demonstrated that 4,6-*O*-isopropylidene-D-glucosaminyl derivatives were highly suitable precursors to residue **D**. (L. A. Mulard, C. Costachel, P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, 19, 849-877; F. Bélot, C. Costachel, K. Wright, A. Phalipon, L. A. Mulard, *Tetrahedron. Lett.* **2002**, 43, 8215-8218) Next, conventional acetylation of **207** gave the required thioglycoside donor **208**.

*L-Rhamnose units (A, B)*: Previous work in the series was mostly based on the use of the 2-*O*-acetyl trichloroacetimidate rhamnopyranosyl donor **213**. (C. Costachel, P. J. Sansonetti, L. A. Mulard, *J. Carbohydr. Chem.* **2000**, 19, 1131-1150; F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, 69, 1060-

1074) Condensation yields were excellent. However, the acetyl protecting group is not fully orthogonal to the benzoyl one, which is a weak point in the strategy since selective de-*O*-acetylation is required twice. The levulinate on the contrary is fully orthogonal to either benzyl or allyl ethers, and to benzoates. The 2-*O*-levulinoyl trichloroacetimidate donor **212** was thus evaluated as an alternative to **213**. It was prepared from the known allyl rhamnopyranoside **209** (P. Westerduin, P. E. der Haan, M. J. Dees, J. H. van Boom, *Carbohydr. Res.* **1988**, *180*, 195-205) in three steps (Figure 9). Indeed, treatment of **209** with levulinic acid gave the fully protected **210** (95%), deallylation of which proceeded in two steps based on (i) isomerisation of the allyl group into the prop-1-enyl ether using an iridium complex, (J. J. Oltvoort, C. A. A. van Boeckel, J. H. der Koning, J. van Boom, *Synthesis* **1981** 305-308) and (ii) subsequent oxidative cleavage of the latter to give the hemiacetal **211** (85-95%). (M. A. Nashed, L. Anderson, *J. Chem. Soc. Chem. Commun.* **1982** 1274-1282) Reaction of the latter with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) resulted in the required donor **212** (95%).

*Synthesis of the pentasaccharide 201 (Figure 10):* The known allyl glycoside **214**, acting as an EC acceptor, temporarily protected at the anomeric position and having a participating group at position 2<sub>C</sub>, was prepared as described in 63% yield from allyl 2,3-*O*-isopropylidene- $\alpha$ -L-rhamnopyranoside. (F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) Its condensation with the trichloroacetimidate donor **212**, performed in the presence of a catalytic amount of TMSOTf, afforded the fully protected trisaccharide **215** (80-95%), and subsequently the known B(E)C acceptor **216** (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074) upon selective removal of the *O*-levulinoyl group with hydrazine hydrate (80-94%). Starting from **216**, this two-step process was repeated to give first the fully protected **217** (54-90%), then the known AB(E)C acceptor **218** (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074) in 80-94% yield. Considering that selective deblocking at positions 2<sub>B</sub> and 2<sub>A</sub> was completed in overnight runs instead of the 5 days required for each corresponding chemoselective *O*-deacetylation steps, the use of the 2-*O*-levulinoyl donor **212** appeared as a suitable alternative to that of **213**. Using a mixture of NIS and triflic acid as the promoter, condensation of the tetrasaccharide acceptor **218** with the thioglycoside donor **208** gave the key intermediate **219** in 58% yield. Although alternative conditions in terms of promoters and solvents (not described) were tested, this rather low yield could not be improved. Bu<sub>3</sub>SnH mediated radical dechlorination of **219** in the presence of a catalytic amount of AIBN readily afforded the corresponding acetamido key intermediate **201** (74%). On one hand, compound **201** may be efficiently converted to the acceptor building block **202** under Zemplén conditions. On the other hand, it was smoothly deallylated into the hemiacetal **220**, following a two-step process as described above. Next, treatment of **220** with

trichloroacetonitrile and DBU allowed its conversion to the building block 3 (82% from 201).

C- Convergent synthesis of the decasaccharide  
D'A'B'(E')C'DAB(E)C

5                   Considering its dimeric nature, a convergent synthetic strategy to the target methyl glycoside of the decasaccharide **D'A'B'(E')C'DAB(E)C (301)** was considered. Indeed, retrosynthetic analysis, supported by previous work in the field,(Bélot, F.; Costachel, C.; Wright, K.; Phalipon, A.; Mulard, L. A. *Tetrahedron. Lett.* **2002**, 43, 8215-8218; Kochetkov, N. K.; Byramova, N. E.; Tsvetkov, Y. E.; Backinovskiy, L. V. *Tetrahedron* **1985**, 41, 3363-3375 ; Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Org. Chem.* **1989**, 54, 2650-2656 ; Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Chem. Soc. Perkin Trans. 1* **1990**, 293-299) indicated that disconnections at the C-D linkage, thus based on two **DAB(E)C** branched pentasaccharides corresponding to a frame-shifted repeating unit **I**, would be the most advantageous (Figure 11). Such a strategy would involve a pentasaccharide acceptor easily derived from the known methyl glycoside **302** (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, 19, 1131-1150) or from the corresponding *N*-acetylated analogue **303** and a pentasaccharide donor bearing a 2-*O*-acyl protecting group at the reducing residue (C) in order to direct glycosylation towards the desired stereochemistry. Depending on the nature of the 2-*N*-acyl group in residue **D**, the latter could derive from the allyl glycosides **304** or **305**. Besides, bearing in mind that the major drawbacks of the linear synthesis of pentasaccharide **302** reported so far (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, 19, 1131-1150) dealt with the selective deblocking of key hydroxyl groups to allow further chain elongation, we describe herein various attempts at a convergent synthesis of the fully protected **DAB(E)C** pentasaccharide as its methyl (**302**, **303**) or allyl (**304**, **305**) glycosides. Precedents concerning a related serotype of *S. flexneri* have indicated that disconnection at the D-A linkage should be avoided (Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Org. Chem.* **1989**, 54, 2650-2656 ; Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Chem. Soc. Perkin Trans. 1* **1990**, 293-299). To our knowledge, disconnection at the B-C linkage was never attempted in the series. However, disconnection at the A-B linkage, based on the use of a combination of a bromide disaccharide donor and Hg(CN)<sub>2</sub>/HgBr<sub>2</sub> as the promoter, was reported once.(N. K. Kochetkov, N. E. Byramova, Y. E. Tsvetkov, L. V. Backinovskiy, *Tetrahedron* **1985**, 41, 3363-3375) In the latter case concerning the synthesis of the linear **DABC** tetrasaccharide, the condensation of two disaccharide building blocks was found more effective than the stepwise strategy. Both routes were considered in the following study. The nature of the repeating unit **I** indicated that any blockwise synthesis involving such linkages would rely on donors lacking any participating group at position 2 of the

reducing residue, thus the relevance of this strategy may be questioned. Nevertheless, although  $\beta$ -glycoside formation was observed occasionally, (Srivastava, O. P.; Hindsgaul, O. *Can. J. Chem.* **1986**, *64*, 2324-2330) the good  $\alpha$ -stereoselectivity reported on several occasions in the literature for glycosylation reactions based on mannosyl donors (Ogawa, T.; Kitajima, T.; Nukada, T. *Carbohydr. Res.* **1983**, *123*, c5-c7 ;Ogawa, T.; Sugimoto, M.; Kitajima, T.; Sadozai, K. K.; Nukada, T. *Tetrahedron Lett.* **1986**, *27*, 5639-5742) and derivatives such as perosaminyl analogues (Lei P.S; Ogawa, Y; Kovac,P. *Carbohydr. Res.* **1996**, *281*, 47-60; Kihlberg, J.; Eichler, E.; Bundle, D. R. *Carbohydr. Res.* **1991**, *211*, 59-75 ; Peters, T.; Bundle, D. R. *Can. J. Chem.* **1989**, *67*, 491-496) or rhamnopyranosyl donors that were either glycosylated at C-2 (Reimer, K. B.; Harris, S. L.; Varma, V.; Pinto, B. M. *Carbohydr. Res.* **1992**, *228*, 399-414), or blocked at this position with a non participating group (Varga, Z.; Bajza, I.; Batta, G.; Liptak, A. *Tetrahedron Lett.* **2001**, *42*, 5283-5286), encouraged the evaluation of the above mentioned block strategies. To follow up the work developed thus far in the *S. flexneri* 2a series, emphasis was placed on the use of the use of trichloroacetimidate (TCA) chemistry (Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21-123).

*Strategy based on the disconnection at the A-B linkage (Figure 11, route a):* Such a strategy involves the coupling of suitable **DA** donors to an appropriate **B(E)C** acceptor. Taking into account the glycosylation chemistry, two sets of disaccharide building blocks (**306**, **307**, **308**), easily obtained from known monosaccharide precursors which were readily available by standard protecting group/activation strategies, were selected (Figure 11). Thus, condensation of the allyl rhamnopyranoside **314**, (Westerduin, P.; der Haan, P. E.; Des, M. J.; van Boom, J. H. *Carbohydr. Res.* **1988**, *180*, 195-205) as precursor to residue **A**, with the glucosaminyl trichloroacetimidate **316**, (Blatter, G.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1994**, *260*, 189-202) as precursor to residue **D**, was performed in the presence of a catalytic amount of TMSOTf to give the fully protected disaccharide **317** (99%). Selective deallylation of **317** proceeded in two steps involving (i) iridium(I)-catalysed isomerisation of the allyl glycoside into the corresponding 1-*O*-propenyl glycoside (Oltvoort, J. J.; van Boeckel, C. A. A. ; der Koning, J. H. d.; van Boom, J. *Synthesis* **1981**, 305-308) and (ii) hydrolysis of the latter (Gigg, R.; Warren, C. D. *J. Chem. Soc. C* **1968**, 1903-1911 ; Gigg, R.; Payne, S.; Conant, R. *J. Carbohydr. Chem.* **1983**, *2*, 207-223). The resulting hemiacetal **318** (81%) was converted into the trichloroacetimidate **306** (78%) by treatment with trichloroacetonitrile in the presence of a catalytic amount of DBU (Figure 12). Knowing from previous experience that conversion of the trichloroacetamide moiety at position 2 of residue **D** (2<sub>D</sub>-*N*-trichloroacetyl) into the required 2<sub>D</sub>-*N*-acetyl group could be somewhat low-yielding, we took advantage of the blockwise approach to perform the above-mentioned transformation at an early stage in the synthesis. Thus, the disaccharide intermediate **317** was converted to the corresponding **319**

(90%) upon overnight treatment with a saturated ammonia methanolic solution and subsequent peracetylation. Conversion of **319** into the hemiacetal **320** (69%), and next into the required trichloroacetimidate donor **307** (86%), followed the procedure described above for the preparation of **306** from **317**. Where glycosylation is concerned, the bifunctional role of thioglycosides as protected acceptors and masked donors is highly appreciated. (S. Oscarson, *Carbohydrates in chemistry and biology. Part 1: Chemistry of saccharides* 2000, 2, 93) Thus, the thiophenyl disaccharide **308** was considered as a possible alternative to the use of the more reactive trichloroacetimidates **306** and **307**. It was synthesized in 97% yield by condensing the known thiophenyl rhamnopyranoside **315** (Lau, R.; Schuele, G.; Schwaneberg, U.; Ziegler, T. *Liebigs Ann. Org. Bioorg. Chem.* 1995, 10, 1745-1754) and **316** in the presence of a catalytic amount of TMSOTf (Figure 12). To fulfil the requirements of the synthesis of **301**, two different trisaccharide building blocks were used, namely either the known methyl glycoside **309** (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* 2000, 19, 1131-1150) or the corresponding allyl glycoside **310**, obtained from the known 2<sub>B</sub>-O-acetylated trisaccharide **342** (see below and Figure 15) (Segat, F.; Mulard, L. A. *Tetrahedron: Asymmetry* 2002, 13, 2211-2222). Condensation of the trisaccharide acceptor **309** and the trichloroacetimidate donor **306** was attempted under various conditions of solvent, temperature and promoter. The  $\alpha$ -linked condensation product, i.e. the known pentasaccharide **302**, (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* 2000, 19, 1131-1150) was at best isolated in 41% yield providing that the glycosylation reaction was run in acetonitrile in the presence of a catalytic amount of TMSOTf, following the inverted procedure protocol (Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* 1991, 32, 3353-3356; Bommer, R.; Kinzy, W.; Schmidt, R. R. *Liebigs Ann. Chem.* 1991, 425-433) to minimize degradation of the donor. Although the  $\alpha$ -selectivity of the glycosylation reaction was good, yields of pentasaccharide remained low, and, as anticipated, use of the alternate trichloroacetimidate donor **307** to give **303** did not result in any improvement (not described). Rearrangement of the activated donor into the corresponding inert trichloroacetamide was observed previously in glycosylation reactions based on trichloroacetimidate donors lacking a participating group at position 2 of the reducing residue. (K. H. Sadozai, T. Nukada, Y. Ito, Y. Nakahara, T. Ogawa, *Carbohydr. Res.* 1986, 157, 101-123) Although the expected side-product was not isolated in any of the attempted glycosylation with **306** or **307**, it was anticipated that the use of an alternate glycosylation chemistry would prevent such side-reaction, and possibly favour the condensation. However, reaction of thiophenyl donor **308** and acceptor **310** in the presence of *N*-iodosuccinimide and catalytic triflic acid did not prove any better as it resulted in mixtures of products from which the target **304** was isolated in very low yield, 10% at best. This strategy was thus not considered any further.

*Strategy based on the disconnection at the B-C linkage (Figure 11, route b).* It was hypothesized that the good  $\alpha$ -selectivity, but poor yields, of the condensation of the various **DA** donors with the **B(E)C** acceptors **309** and **310** might result from the poor nucleophilicity of the axial hydroxyl at position 2<sub>B</sub>. Thus, we next turned to the 3<sub>C</sub>-OH as a possible elongation site in the design of a block synthesis of pentasaccharide **305**. Considering such a disconnection approach suggests the use of a **DAB** trisaccharide donor for coupling to an **EC** disaccharide acceptor. As the target pentasaccharide should serve as an appropriate donor in the construction of **301**, we reasoned that an acyl participating group had to be present at its position 2<sub>C</sub>. Thus, two 2<sub>C</sub>-*O*-acylated **EC** building blocks, **311** or **312**, were considered. In order to avoid any unnecessary deprotection step at the pentasaccharide level, the trisaccharide **313**, bearing an acetamido functionality at position 2<sub>D</sub>, was selected as the donor. Indeed, as it involves the less readily available **EC** structure in fewer synthetic steps and does not rely on selective deprotection at the 2<sub>A</sub> position, this path was found particularly attractive. Again, it relies on the use of appropriately functionalized known monosaccharide intermediates (Figure 13).

The known key di-rhamnoside core structure **322** (Zhang, J.; Mao, J. M.; Chen, H. M.; Cai, M. S. *Tetrahedron: Asymmetry* **1994**, *5*, 2283-2290) was formed by glycosylation of the allyl rhamnoside **314** with the trichloroacetimidate donor **321** (Castro-Palomino, J. C.; Rensoli, M. H.; Bencomo, V. V. *J. Carbohydr. Chem.* **1996**, *15*, 137-146) in the presence of a catalytic amount of TMSOTf. It should be pointed out that using diethyl ether as the solvent, the isolated yield of **322** was 92%, which compares favourably with those obtained previously, 60% and 76.2% (Zhang, J.; Mao, J. M.; Chen, H. M.; Cai, M. S. *Tetrahedron: Asymmetry* **1994**, *5*, 2283-2290), when running the reaction in dichloromethane under promotion by TMSOTf or BF<sub>3</sub>·OEt<sub>2</sub>, respectively. De-*O*-acetylation under Zemplén conditions afforded the 2<sub>A</sub>-*O*-unprotected acceptor **323** (Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Org. Chem.* **1989**, *54*, 2650-2656) in 93% yield.

As shown previously in the construction of the **DA** intermediate **317**, the *N*-trichloroacetyl trichloroacetimidate **316** appears to be a highly suitable precursor to residue **D** when involved in the formation of the  $\beta$ -GlcNAc linkage at the poorly reactive 2<sub>A</sub> position. Indeed, reaction of **316** with the acceptor **323** in 1,2-dichloroethane in the presence of TMSOTf went smoothly and gave the trisaccharide **325** in 96% yield. However, conversion of the *N*-trichloroacetyl group to the *N*-acetyl derivative **327** was rather less successful as the desired trisaccharide was obtained in only 42 % yield when treated under conditions that had previously been used in the case of a related oligosaccharide (sodium methoxide, Et<sub>3</sub>N, followed by re-*N,O*-acetylation). (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150). This result led us to reconsider the protection pattern of the glucosamine donor. The *N*-



tetrachlorophthalimide group has been proposed as an alternative to overcome problems associated with the widely spread phthalimido procedure when introducing a 2-acetamido-2-deoxy- $\beta$ -D-glucopyranosidic linkage (Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302-3303). Thus, the *N*-tetrachlorophthalimide trichloroacetimidate donor **324** was selected as an alternative. It was prepared as described from commercially available D-glucosamine (Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36*, 5343-5346), apart from in the final imidate formation step, where we found the use of potassium carbonate as base to be more satisfactory than DBU. Glycosylation of **323** with **324** in the presence of TMSOTf resulted in the trisaccharide **328** in 65% yield. The tetrachlorophthaloyl group was then removed by the action of ethylenediamine, and subsequent re-*N,O*-acetylation gave the trisaccharide **327** in 65% yield. The latter was next converted into the donor **313** in two steps, analogous to those described for the preparation of **306** from **317**. Indeed, de-*O*-allylation of **327** cleanly gave the hemiacetal **329** (83%), which was then activated into the required trichloroacetimidate (94%). It is worth mentioning that although they involve a different **D** precursor, both strategies give access to the intermediate **327** in closely related yields, 40 and 42%, respectively.

Initial attempts to form the pentasaccharide **305** from **313** and the previously described acceptor **311** (Segat, F.; Mulard, L. A. *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) in the presence of TMSOTf as promoter were rather unsuccessful, resulting in at best 17% of the desired product, accompanied by decomposition of the donor into the hemiacetal **329** (75%). By using  $\text{BF}_3 \cdot \text{OEt}_2$  as the promoter in place of TMSOTf, reaction of **311** with **313** at room temperature provided **305** in 44% yield, with the acceptor **311** and hemiacetal **329** also recovered in 54% and 29% yield, respectively. We considered that the poor reactivity of the acceptor was responsible for these results, as since the  $^{13}\text{C}$  NMR of **305**, showing several distorted signals (notably C-1<sub>B</sub>, as well as most certainly C-3<sub>C</sub> and C-4<sub>C</sub>), suggests restricted conformational flexibility around the position 3<sub>C</sub>. For that matter, the 2<sub>C</sub>-*O*-acetylated disaccharide **312** was considered as an alternate acceptor. Analogously to the preparation of **311**, it was obtained from the known diol **330** through regioselective opening of the intermediate orthoester. However, coupling of the potentially less hindered acceptor **312** and the trisaccharide donor **313** resulted, at best, in the isolation of the condensation product **331** in 42% yield (not described).

The modest yield of **305** and **331** obtained by this route made the alternative reaction path (Figure 14) worth investigating, despite the more numerous synthetic steps required. Indeed, it was found rather appealing when evaluated independently in a closely related series (unpublished results). By this route, a tetrasaccharide acceptor can be formed from two disaccharide building blocks (**EC** and **AB**), and coupled with an appropriate monosaccharide donor as precursor to **D**.

Considering that selective deprotection of the 2<sub>A</sub> hydroxyl group would occur in the course of the synthesis, glycosylation attempts were limited to the 2-*O*-benzoylated acceptor **311**. The disaccharide donor necessary for this path could be derived from the building block **323**, already in hand. The choice of temporary protecting group at position 2<sub>A</sub> was determined by our experience of the stepwise synthesis of the corresponding methyl pentasaccharide, (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150) where we noted that an acetate group at this position may not be fully orthogonal to the benzoate located at position 2<sub>C</sub>. The chosen group had also to support removal of the anomeric allyl group and the subsequent conversion to the trichloroacetimidate. At first, a chloroacetate group was anticipated to fulfil these requirements. Thus, the disaccharide **323** was treated with chloroacetic anhydride and pyridine to give the derivative **332** (57%). Anomeric deprotection to give the hemiacetal **333** (84%) and subsequent trichloroacetimidate activation of the latter into the donor **334** (83%) were performed in the same way as before. Coupling of **311** with **334**, carried out in the presence of TMSOTf at -40°C, yielded a complex mixture of products. When the temperature was lowered to -60°C, the condensation product **338** could be isolated in 22% yield. Alternative donor protection was attempted. Treatment of **323** with *p*-methoxybenzyl chloride and sodium hydride gave the fully protected derivative **335** (97%), which was cleanly converted into the trichloroacetimidate donor **337** (82%) in two steps involving the hemiacetal intermediate **336** (73%). Glycosylation of **311** with **337** in the presence of TMSOTf at -40°C gave the desired tetrasaccharide **339** in 44% yield. When the temperature was lowered to -60°C, the yield of **339** fell to 34% and a second major product **340** (21%) was observed in the mixture. Indeed, examination of the NMR spectra of this product revealed that the *p*MeOBn group had been lost. That **340** was the acceptor required for the next step brought the estimated yield of condensation to 55%. Nevertheless, the overall outcome of this blockwise strategy did not match our expectations, and this route was abandoned.

*Linear strategy to the fully protected pentasaccharide 304 (Figure 15):*

As preliminary studies have demonstrated, rapid access to suitable building blocks allowing the synthesis of higher-order oligosaccharides representative of fragments of the O-SP of *S. flexneri* 2a remains a challenge. Major conclusions drawn from our studies favour the design of a linear synthesis of the target **304**. Indeed, when put together with our previous work, such as the synthesis of tetrasaccharide **341** (95%) (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150) or that of trisaccharide **342** (97%) (Segat, F.; Mulard, L. A. *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222), all the above-described attempted couplings outlined the loss of efficiency of glycosylation reactions involving rhamnopyranosyl donors glycosylated at position 2 in comparison to those involving the corresponding acetylated donor. Thus, matching the

linear strategy of the methyl pentasaccharide **2** described previously, (Costachel, C Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150) a synthesis of **304**, based on donors bearing a participating group at O-2, was designed. Three key building blocks were selected. These were the readily accessible EC disaccharide acceptor **311** benzoylated at C-2 as required for the final condensation step leading to the full protected decasaccharide intermediate; the rhamnopyranosyl trichloroacetimidate **321** which serves as a precursor to residues **A** and **B**, and bears a both temporary and participating group at position 2; and the trichloroacetamide glucosaminyl donor **316** as a precursor to residue **D**. As stated above, coupling of **311** and **321** gave **342** in high yield. As observed in the methyl glycoside series, (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150) de-O-acetylation using MeONa or methanolic HCl was poorly selective. Although, guanidine/guanidinium nitrate was proposed as a mild and selective O-deacetylation reagent compatible with the presence of benzoyl protecting groups, (Ellervik, U.; Magnusson, G. *Tetrahedron Lett.* **1997**, *38*, 1627-1628) none of the conditions tested prevented partial debenzoylation leading to diol **343**, as easily confirmed from NMR analysis (not described). The required alcohol **310** was readily obtained in an acceptable yield of 84% yield by a five-day acid catalysed methanolysis, using HBF<sub>4</sub> in diethyl ether/methanol, (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150 ; Pozsgay, V.; Coxon, B. *Carbohydr. Res.* **1994**, *257*, 189-215) of the fully protected intermediate **342**. Repeating this two-step process using **310** as the acceptor and **321** as the donor resulted first in the intermediate **344** (90%), and next in the tetrasaccharide acceptor **340** (84%). Glycosylation of the latter with **316** gave the fully protected pentasaccharide **304** in high yield (98%), thus confirming that the combination of the trichloroacetamide participating group and the trichloroacetimidate activation mode in **316** results in a potent donor to be used as a precursor to residue **D** in the *S. flexneri* series, where low-reactive glycosyl acceptors are concerned. Following the above described procedure, selective anomeric deprotection of **304** furnished the hemiacetal **345** which was smoothly converted to the trichloroacetimidate donor **346** (66% from **304**). From these data, the linear synthesis of **34**, truly benefiting from the use of **321** as a common precursor to residue **A** and **B**, appears as a reasonable alternative to the block syntheses which were evaluated in parallel.

*Synthesis of the target decasaccharide 301:* Having a pentasaccharide donor in hand, focus was next placed on the synthesis of an appropriate pentasaccharide acceptor. In our recent description of the convergent synthesis of the B'(E')C'DAB(E)C octasaccharide, (F. Bélot, C. Costachel, K. Wright, A. Phalipon, L. A. Mulard, *Tetrahedron. Lett.* **2002**, *43*, 8215-8218) the pentasaccharide **348**, bearing a 4<sub>D</sub>,6<sub>D</sub>-O-isopropylidene protecting group, was found a most convenient acceptor which encouraged its selection in the present work. Briefly, **348** was prepared in two steps from the known

302. Thus, mild transesterification of 302 under Zemplén conditions allowed the selective removal of the acetyl groups to give triol 347, which was converted to the required acceptor 348 (72% from 302) upon subsequent treatment with 2-methoxypropene. Relying on previous optimisation of the glycosylation step (Bélot, F.; Costachel, C.; Wright, K. Phalipon, A.; Mulard, L. A. *Tetrahedron. Lett.* **2002**, *43*, 8215-8218), the condensation of 348 and 346 was performed in the presence of a catalytic amount of triflic acid. However, probably due to the closely related nature of the donor and acceptor, the reaction resulted in an inseparable mixture of the fully protected 349 and the hemiacetal 345 resulting from partial hydrolysis of the donor. Most conveniently, acidic hydrolysis of the mixture allowing the selective removal of the isopropylidene group in 349, gave the intermediate diol 350 in a satisfactory yield of 72% for the two steps. According to the deprotection strategy used for the preparation of the closely related octasaccharide (Bélot, F.; Costachel, C.; Wright, K.; Phalipon, A.; Mulard, L. A. *Tetrahedron. Lett.* **2002**, *43*, 8215-8218), diol 350 was engaged in a controlled de-*O*-acylation process upon treatment with hot methanolic sodium methoxide. However, partial cleavage of the trichloroacetyl moiety, leading to an inseparable mixture, was observed which prevented further use of this strategy. Indeed, it was assumed that besides being isolated and therefore resistant to Zemplén transacetylation conditions (Liptak, A.; Szurmai, Z.; Nanasi, P.; Neszmelyi, A. *Carbohydr. Res.* **1982**, *99*; Szurmai, Z.; Liptak, A.; Snatzke, G. *Carbohydr. Res.* **1990**, *200*, 201-208; Szurmai, Z.; Kerékgyarto, J.; Harangi, J.; Liptak, A. *Carbohydr. Res.* **1987**, *174*, 313-325), the 2-*O*-benzoyl groups were most probably highly hindered which contributed to their slow deprotection. Alternatively, 350 was submitted to an efficient two-step in-house process involving first, hydrogenolysis under acidic conditions which allowed the removal of the benzyl groups and second, basic hydrochlorination which resulted in the conversion of the *N*-trichloroacetyl groups into the required *N*-acetyl ones, thus affording 352. Subsequent transesterification gave the final target 301 in 37% yield from 350 (Figure 16).

**D- Synthesis of the 2-aminoethyl glycoside of a hapten representative of the O-specific polysaccharide of *Shigella flexneri* serotype 2a and of a corresponding PADRE-conjugate**

Studies on the recognition of synthetic fragments of the O-SP by protective homologous monoclonal antibodies suggested that sequences larger than one repeating unit were more antigenic, thus probably better mimicking the natural polysaccharide than shorter ones. Indeed, it is anticipated that better mimics of the O-SP, in terms of both antigenicity and conformation, would lead to conjugates of higher immunogenicity. For that reason, the preparation of conjugates comprising oligosaccharides larger than one repeating unit was undertaken.

We report herein on the synthesis of the 2-aminoethyl glycosides of a hexasaccharide (**402**) and on that of the corresponding fully synthetic conjugate (**401**) using the PADRE as a universal T-helper peptide (see section E for the background). We have demonstrated that disconnection at the C-D linkage was appropriate for the construction of large fragments of the *S. flexneri* 2a O-SP (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074). Based on our experience in the field, a strategy to target **401**, implicating the DAB(E)C building block bearing the required acetamido function at position 2<sub>D</sub> (**406**) as donor and the recently disclosed acceptor **405** (K. Wright, C. Guerreiro, I. Laurent, F. Baleux, L. A. Mulard, *Org. Biomol. Chem.* **2004**, *2*, 1518-1527) as a precursor to the spacer-armed D residue (Figure 17). Although permanent blocking of OH-4<sub>D</sub> and OH-6<sub>D</sub> with an isopropylidene acetal may appear somewhat unusual, this choice was a key feature of the strategy. It was based on former observations in the methyl glycoside series, demonstrating that its use could overcome some of the known drawbacks of the corresponding benzylidene acetal, (Bundle, D. R.; Josephson, S. *Can. J. Chem.* **1979**, *57*, 662-668 ; Mulard, L. A.; Costachel, C.; Sansonetti, P. J. *J. Carbohydr. Chem.* **2000**, *19*, 849-877) including its poor solubility.

*Synthesis of the hexasaccharide 402 (Figure 18):* The key pentasaccharide donor **406** was obtained from the recently disclosed precursor **407** (see section F, compound **611**). The latter was converted to the hemiacetal **408** following a two-step process including Iridium complex promoted isomerisation of the allyl moiety into the propen-1-yl, (Oltvoort, J. J.; van Boeckel, C. A. A.; der Koning, J. H.; van Boom, J. *Synthesis* **1981**, 305-308) and hydrolysis of the latter upon treatment with aqueous iodine (Nashed, M. A.; Anderson, L. *J. Chem. Soc. Chem. Commun.* **1982**, 1274-1282). Subsequent reaction of **408** with trichloroacetonitrile in the presence of catalytic 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) cleanly gave the trichloroacetimidate donor **406** (85% from **407**). Previous glycosidation attempts in the series indicated that when run at low temperature or room temperature, reactions using the D acceptor **405** occasionally resulted in a rather poor yield of the condensation product. This was tentatively explained by the still rather poor solubility of **405**. When using 1,2-dichloroethane (1,2-DCE) as the solvent, the condensation could be performed at higher temperature, which proved rewarding. Indeed, optimized coupling conditions relied on the concomitant use of a catalytic amount of triflic acid in the presence of 4Å molecular sieves as the promoter and 1,2-DCE as the solvent, while the condensation was performed at 80°C. The fully protected hexasaccharide **409** was isolated in a satisfactory 78% yield. That the hemiacetal **408**, resulting from the hydrolysis of the excess donor could be recovered was of great advantage is one considers scaling up the process (not described). Acidic hydrolysis of the isopropylidene acetal smoothly converted **409** into the corresponding diol **410** (94%). Resistance of isolated benzoyl groups to Zemplén transesterification has been reported

(Lipták, A.; Szurmai, Z.; Nanasi, P.; Neszmelyi, A. *Carbohydr. Res.* **1982**, *99*, 13-21, Szurmai, Z.; Kerékgyarto, J.; Harangi, J.; Lipták, A. *Carbohydr. Res.* **1987**, *174*, 313-325. Szurmai, Z.; Lipták, A.; Snatzke, G. *Carbohydr. Res.* **1990**, *200*, 201-208). It was also observed previously in the series, upon attempted removal of a benzoyl group located at position 2<sub>C</sub> (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074). Again, the 2<sub>C</sub>-*O*-benzoyl group in **410** was particularly resistant to Zemplén de-*O*-acylation, and successful transesterification required a week. In that case heating was avoided in order to prevent any potential migration of the acyl group which would lead to the *N*-deacylated product. Conversion of the hexaol **411** into the target **402** (77%) was successfully accomplished upon concomitant hydrogenolysis of the remaining benzyl protecting group and reduction of the azido moiety into the corresponding amine. As observed earlier, the latter was best performed under acidic conditions.

*Synthesis of the fully synthetic glycoconjugate 401 (Figure 17):* 4-(*N*-maleimido)-*n*-butanoyl was selected as the linker, and incorporated using commercially available **404** by covalent linkage to the side chain amino group of a Lysine residue added at the C-terminus of the PADRE sequence (PADRE-Lys). The latter was assembled using standard Fmoc chemistry for solid-phase peptide synthesis (Chan, W. C.; White, P. D. *Fmoc solid phase peptide synthesis*; Oxford University Press: New York, 2000). Standard side chain protecting groups were used, except for that of the C-terminal Lysine side chain which was protected by the 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) group (Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan W. C. *Tetrahedron Lett.* **1998**, *39*, 1603-1606) to allow specific introduction of the maleimide group. The thiol functionality was introduced onto the carbohydrate haptens as a masked thiol function (acetylthioester), which is easily generated *in situ* during the conjugation process. Thus, reaction of **402** with S-acetylthioglycolic acid pentafluorophenyl ester (SAMA-oPfp) resulted in the site-selective elongation of the aminoethyl spacer via a thioacetyl acetamido linker. Derivatization could be monitored by RP-HPLC with detection at 215 nm. Under these conditions, the required thioacetyl-armed intermediate **412** was isolated in 53% yield. Its structure was confirmed based on MS and NMR analysis. Conjugation of the carbohydrate haptens to the maleimido activated PADRE-Lys (**403**) was run in phosphate buffer at pH 6.0 in presence of hydroxylamine (H. F. Brugghe, H. A. M. Timmermans, L. M. A. van Unen, G. J. T. Hove, G. W. der Werken, J. T. Poolman, P. Hoogerhout, *Int. J. Peptide Protein Res.* **1994**, *43*, 166) and monitored by RP-HPLC. Lastly, RP-HPLC purification gave the target neoglycopeptide **401** as a single product, whose identity was assessed based on MS analysis, in yields of 58%.

**E- Preparation of chemically defined glycopeptides as potential synthetic conjugate vaccines against *Shigella flexneri* serotype 2a disease**

The target neoglycopeptides were constructed by covalently linking a short peptide, serving as a T-helper epitope, to appropriate carbohydrate haptens, serving as B epitopes mimicking the *S. flexneri* 2a O-Ag. Our approach is based on a rational base involving a preliminary study of the interaction between the bacterial O-SP and homologous protective monoclonal antibodies, which helped to define the carbohydrate haptens.

Fragments **ECD**, **B(E)CD** and **AB(E)CD** were selected as haptens that will act as B-epitopes in the conjugates. Three fully synthetic linear neoglycopeptides **501**, **502** and **503**, corresponding to haptens **ECD**, **B(E)CD**, and **AB(E)CD**, respectively, were synthesized according to a strategy built up on the concept of chemoselective ligation which allows the selective one-point attachment of the free B and T epitopes in aqueous media. All conjugates involve the peptide PADRE (J. Alexander, J. Sidney, S. Southwood, J. Ruppert, C. Oseroff, A. Maewal, K. Snoke, H. M. Serra, R. T. Kubo, A. Sette, H. M. Grey, *Immunity* **1994**, *1*, 751-761; J. Alexander, A.-F. d. Guercio, A. Maewal, L. Qiao, J. Fikes, R. W. Chesnut, J. Paulson, D. R. Bundle, S. DeFrees, A. Sette, *J. Immunol.* **2000**, *164*, 1625-1633) as the universal T-cell epitope.

*Retrosynthetic analysis of the saccharidic haptens (Figure 19):* Analysis of *S. flexneri* 2a O-SP suggests that, due to the 1,2-cis glycosidic linkage involved in the construction of the EC disaccharide is probably the most demanding. Besides, prior work in this laboratory has shown that the C-D glycosidic linkage was an appropriate disconnection site when dealing with the blockwise synthesis of oligosaccharide fragments of *S. flexneri* O-2a SP. (F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) These observations supported the design of a synthetic strategy common to all three targets. Basically, it relies on (i) the condensation of an **EC** (**504**), (C. Costachel, P. J. Sansonetti, L. A. Mulard, *J. Carbohydr. Chem.* **2000**, *19*, 1131-1150) **B(E)C** (**505**) (F. Bélot, C. Costachel, K. Wright, A. Phalipon, L. A. Mulard, *Tetrahedron. Lett.* **2002**, *43*, 8215-8218) or **AB(E)C** (**506**) donor to a **D** acceptor (**507**), functionalized at the anomeric position with an azidoethyl spacer; (ii) elongation of the spacer with introduction of a masked thiol group to allow its coupling onto a PADRE peptide derivatized by a maleimido group on a C-terminal Lysine (**508**). The carbohydrate synthesis relies on the trichloroacetimidate methodology and the use of known building blocks whenever possible.

*Synthesis of the aminoethyl ECD building block 518 (Figure 20):* The now easily accessible disaccharide donor **504**, with a benzoyl participating group at position 2<sub>C</sub>, was used as the precursor to the **EC** moiety in the construction of **501**. It was prepared, as described, (Costachel, C.; Sansonetti, P. J.; Mulard, L. A. *J. Carbohydr.*

*Chem.* **2000**, *19*, 1131-1150) in 5 steps and 45% overall yield from 2,3,4,6-tetra-*O*-benzyl- $\beta$ -D-glucopyranosyl trichloroacetimidate (**509**)(R. R. Schmidt, J. Michel, M. Roos, *Liebigs Ann. Chem.* **1984** 1343-1357; R. R. Schmidt, J. Michel, *Tetrahedron Lett.* **1984**, *25*, 821-824) and allyl 2,3-*O*-isopropylidene- $\alpha$ -L-rhamnopyranoside (**510**)(R. Gigg, S. Payne, R. Conant, *J. Carbohydr. Chem.* **1983**, *2*, 207-223) through the key intermediate diol **511** (69% from **510**). Introduction of the azidoethyl spacer on a glucosaminyl intermediate was performed according to a known procedure (Eklind, T.; Gustafsson, R.; Tidén, A.-K.; Norberg, T.; Åberg, P.-M. *J. Carbohydr. Chem.* **1996**, *15*, 1161-1174) by coupling of azidoethanol onto the oxazoline **512** to give the triacetate **513**.(T. Eklind, R. Gustafsson, A.-K. Tidén, T. Norberg, P.-M. Åberg, *J. Carbohydr. Chem.* **1996**, *15*, 1161-1174) We have shown on several occasions in the *S. flexneri* series, that regioselective protection of the 4- and 6-OH groups of precursors to residue **D** with an isopropylidene acetal was appropriate, especially when such precursors are involved in a blockwise synthesis based on the disconnection at the C-D linkage. Thus, Zemplén deacetylation of **513** gave the triol **514** which was converted to the key acceptor **507** (81% from **513**) upon reaction with 2,2-dimethoxypropane under acid catalysis. When the latter was glycosylated with the donor **504** in the presence of BF<sub>3</sub>.OEt<sub>2</sub> in CH<sub>2</sub>Cl<sub>2</sub>, the fully protected trisaccharide **515** was isolated in 58% yield together with the diol **516** (30%), resulting from partial loss of the isopropylidene acetal. When **504** and **507** were glycosylated in the presence of a catalytic amount of TMSOTf, no side-reaction was observed, and the condensation product **515** was obtained in 86% yield. Quantitative conversion of **515** into **516** was more conveniently achieved by acidic hydrolysis of the former with 95% aq TFA. Debenzoylation of **516** gave the tetraol **517** (94%) which was subsequently transformed into the aminoethyl-trisaccharide **518** (69%) by hydrogenation in the presence of palladium-on-charcoal (Pd/C) and 1M aq HCl to convert the formed amine to its hydrochloride salt. Indeed, others have pointed out that hydrogenolysis using Pd/C in the presence of a free amine was sluggish and low-yielding (Stahl, W.; Sprengard, U.; Kretschmar, G.; Kunz, H. *Angew. Chem. Int. Ed.* **1994**, *33*, 2096-2098 ; Spikjer, N. M.; Keuning, C. A.; Hooglugt, M. *Tetrahedron* **1996**, *52*, 5945-5960 ; Li, Q.; Li, H.; Lou, Q.-H.; Su, B.; Cai, M.-S.; Li, Z.-J. *Carbohydr. Res.* **2002**, *337*, 1929-1934). In order to prevent any side-reaction at a latter stage of the synthesis, isolation of pure **518** was subsequently submitted to reversed-phase HPLC (RP-HPLC).

*Synthesis of the aminoethyl B(E)CD building block 525 (Figure 21):* The known rhamnopyranosyl trichloroacetimidate **520**, acetylated at its 2-, 3-, and 4-OH groups thus acting as a chain terminator, was chosen as the precursor to residue **B**. Benzoylation of diol **511** to give **519** was performed by regioselective opening of the cyclic orthoester intermediate as described (Segat, F.; Mulard, L. A. *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222). Glycosylation of the latter by donor **520**, with activation by a catalytic amount



of TMSOTf proceeded smoothly in Et<sub>2</sub>O to yield the fully protected trisaccharide **521** (89%), which was de-*O*-allylated into the hemiacetal **522** (80%) following a two step process involving (i) iridium(I)-catalysed isomerisation of the allyl glycoside to the prop-1-enyl glycoside (Oltvoort, J. J.; van Boeckel, C. A. A.; der Koning, J. H.; van Boom, J. *Synthesis* **1981**, 305-308) and (ii) subsequent hydrolysis (Gigg, R.; Payne, S.; Conant, R. J. *Carbohydr. Chem.* **1983**, 2, 207-223 ; Gigg, R.; Warren, C. D. *J. Chem. Soc. C* **1968**, 1903-1911). The selected trichloroacetimidate leaving group was introduced by treatment of **522** with trichloroacetonitrile in the presence of a catalytic amount of DBU, which resulted in the formation of **505** (99%). Condensation of the latter with acceptor **507** was performed in CH<sub>2</sub>Cl<sub>2</sub> in the presence of a catalytic amount of trifluoromethanesulfonic acid (TfOH) to give the required tetrasaccharide **523** (76%). Acidic hydrolysis of the latter using 95% aq TFA gave the intermediate diol **524** in 95% yield. Deacylation of the resulting diol under Zemplén conditions followed by debenzylation and concomitant conversion of the azide into the corresponding amine to give the key aminoethyl-armed tetrasaccharide **525** (77%) was performed by treatment of **524** with hydrogen in the presence of Pd/C under acidic conditions. Again, compound **525** was purified by RP-HLPC before elongation of the spacer or conjugation.

*Synthesis of the aminoethyl AB(E)CD building block 537 (Figure 22):*

The synthesis of **537** is based on the condensation of acceptor **507** and donor **506**, which resulted from the selective deallylation and anomeric activation of the key intermediate tetrasaccharide **533**. The latter was obtained according to two routes following either a block strategy (route 1) based on the condensation of an AB disaccharide donor (**530**) and the EC disaccharide acceptor **519**, or a linear strategy (route 2) involving the stepwise elongation of **519**. The construction of the donor **530** was based on the use of the known allyl rhamnopyranoside **526** (Westerduin, P.; der Haan, P. E.; Dees, M. J.; van Boom, J. H. *Carbohydr. Res.* **1988**, 180, 195-205), having permanent protecting groups at position 3 and 4, as the precursor to residue B, and the trichloroacetimidate chain terminator **527** (Ziegler, T.; Bien, F.; Jurish, C. *Tetrahedron: Asymmetry* **1998**, 9, 765-780), acting as a precursor to residue A. Condensation of the two entities in the presence of a catalytic amount of TMSOTf resulted in the fully protected **528** (96%), which was selectively de-*O*-allylated into **529** (84%) according to the protocol described above for the preparation of **522**. Subsequent treatment of **529** with trichloroacetonitrile and a catalytic amount of DBU gave the required **530** (96%). Glycosylation of **519** with the latter under TMSOTf promotion afforded the fully protected tetrasaccharide **533** in 55% yield. No β-anomer was detected. Route 1 was considered initially in order to prevent extensive consumption of the EC disaccharide **511**. Given the relatively low yield of coupling of **519** and **530**, route 2 was considered as well. Of all precursors to **534**, only that to residue B, namely the donor and potential acceptor **531**, differed from those used in route 1. Conventional glycosylation

of disaccharide **519** and **531** and subsequent selective deacetylation using methanolic HBF<sub>4</sub>, gave the acceptor **532** in 70% yield from **519**. The trisaccharide **532** was glycosylated with trichloroacetimidate **527** in an analogous fashion to glycosylation of **519** with **530**, yielding **533** (92%). Anomeric de-*O*-allylation of this key intermediate, as described above for the preparation of **522**, gave the corresponding hemiacetal **534** (90%) which was converted into the required trichloroacetimidate **506** (88%) upon treatment with trichloroacetonitrile and DBU. Condensation of donor **506** with the glucosaminyl acceptor **507** was performed under promotion by TfOH or TMSOTf, which resulted in the fully protected pentasaccharide **535** in 62% and 80% yield, respectively. Following the process described for the preparation of **525**, compound **535** was submitted to acetolysis (97%) and subsequent Zemplén deacylation to give the partially deblocked **536** (87%), which was next converted to the aminoethyl-spacer pentasaccharide **537** upon treatment with hydrogen in the presence of Pd/C. Final RP-HPLC purification resulted in the isolation of **537** in 53% yield.

*Synthesis of the target neoglycopeptides 501-503 (Figure 23):* In all cases, chemoselective ligation of the B and T epitopes was achieved through coupling of the carbohydrate haptens pre-functionalized with a thiol function and a maleimido group properly introduced at the C terminus of the T helper peptide. Such a strategy was chosen in order to exploit the high reactivity and specificity of thiol groups towards the maleimide functionality (Marrian, D. H. *J. Chem. Soc. C* **1949**, 1515), which allows specific and high-yielding modification of the former in the presence of other nucleophiles (Hermanson, G. T. *Bioconjugate techniques*; Academic Press: New York, 1996). It was used previously under various forms in the coupling of carbohydrate haptens to either proteins (Ragupathi, G.; Koganty, R. R.; Qiu, D.; Llyod, K. O.; Livingston, P. O. *Glycoconjugate J.* **1998**, *15*, 217-221; Shin, I.; Jung, H.; Lee, M. *Tetrahedron Lett.* **2001**, *42*, 1325-1328) or peptides (Kandil, A.; Chan, N.; Klein, M.; Chong, P. *Glycoconjugate J.* **1997**, *14*, 13-17). To our knowledge, in all the reported cases the maleimide functionality was introduced onto the carbohydrate hapten. On the contrary, our strategy relies on the introduction of this activating group on the T helper peptide. The immunogenicity of various maleimide-derived coupling reagents was evaluated in a model system. Based on the reported data, (Peeters, J. M.; Hazendonk, T. G.; Beuvery, E. C.; Tesser, G. I. *J. Immunol. Methods* **1989**, *120*, 133-143) 4-(*N*-maleimido)-*n*-butanoyl was selected as the linker, and incorporated by covalent linkage to the side chain amino group of a Lysine residue added at the C-terminus of the PADRE sequence (PADRE-Lys). It is worth mentioning that the strategy described herein somewhat differs from that described by others when demonstrating the usefulness of PADRE in the construction of immunogenic neoglycopeptides (Alexander, J.; Guercio, A.-F. d.; Maewal, A.; Qiao, L.; Fikes, J.; Chesnut, R. W.; Paulson, J.; Bundle, D. R.; DeFrees, S.; Sette, A. *J. Immunol.* **2000**, *164*, 1625-1633).

The Lysine-modified PADRE was assembled using standard Fmoc chemistry for solid-phase peptide synthesis (Chan, W. C.; White, P. D. *Fmoc solid phase peptide synthesis*; Oxford University Press: New York, 2000). Standard side chain protecting groups were used, except for that of the C-terminal Lysine side chain which was protected by the 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) group (Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett.* **1998**, *39*, 1603-1606). Indeed, this orthogonal protecting group strategy allows specific introduction of the maleimide group on the C-terminal Lysine, upon selective cleavage of the ivDde by hydrazine. The thiol functionality was introduced onto the carbohydrate haptens as a masked thiol function (acetylthioester), which is easily generated *in situ* during the conjugation process. Thus, reaction of **518**, **525**, and **537** with *S*-acetylthioglycolic acid pentafluorophenyl ester (SAMA- $\alpha$ Pfp) resulted in the site-selective elongation of their aminoethyl spacer via a thioacetyl acetamido linker. Derivatization could be monitored by RP-HPLC with detection at 215 nm. Under these conditions, the required thioacetyl-armed intermediates, **538**, **539** and **540** were isolated in 53%, 74%, and 75% yield, respectively. Their structure was confirmed based on MS and NMR analysis. Conjugation of the carbohydrate haptens to the maleimido activated PADRE-Lys (**508**) was run in phosphate buffer at pH 6.0 in presence of hydroxylamine H.F. Brugghe, H. A. M. Timmermans, L. M. A. van Unen, G. J. T. Hove, G. W. der Werken, J. T. Poolman, P. Hoogerhout, *Int. J. Peptide Protein Res.* **1994**, *43*, 166-172) and monitored by RP-HPLC. Lastly, RP-HPLC purification gave the target neoglycopeptide: **501**, **502**, and **503** as single products, which identity was assessed based on MS analysis, in yields of 58%, 48% and 46%, respectively.

**F- Synthesis of two linear PADRE-conjugates bearing a deca- or pentadecasaccharide B epitope as potential synthetic vaccine against *Shigella flexner* serotype 2a infection**

We report herein on the synthesis of the PADRE conjugates of a deca (**601**) and a pentadecasaccharide (**602**), corresponding to a dimer [AB(E)CD]<sub>2</sub> and a trimer [AB(E)CD]<sub>3</sub> of the branched pentasaccharide **I**, respectively (Figure 24). The synthesis is based on a modular approach involving three partners. Basically, it relies on (i) the use of appropriate haptens functionalized at the anomeric position with an aminoethyl spacer, **603** and **604**, respectively; (ii) the incorporation of a thioacetyl acetamido linker as a masked thiol functionality, and (iii) the use of a PADRE peptide derivatized by a maleimido group on a C-terminal lysine (**605**).

Considering the targets **603** and **604**, a disconnection at the D-A linkage would appear most appropriate. However, others have shown that such a disconnection strategy was not suitable even when involving di- or trisaccharide building blocks (B. M. Pinto, K. B. Reimer, D. G. Morissette, D. R. Bundle, *J. Org. Chem.* **1989**, *54*, 2650 ; B. M.

Pinto, K. B. Reimer, D. G. Morissette, D. R. Bundle, *Carbohydr. Res.* **1990**, *196*, 156) thus this route was avoided. More recently, disconnections at the A-B, B-C and C-I linkages were evaluated in this laboratory when synthesizing successfully the methyl glycoside of the frame-shifted decasaccharide **D'A'B'(E')C'DAB(E)C** (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074). I was demonstrated on that occasion that disconnection at the C-D linkage was indeed appropriate for the construction of large fragments of the *S. flexneri* 2a O-SP. Based on our experience in the field, we designed a blockwise strategy to targets **603** and **604** implicating an **AB(E)C** tetrasaccharide donor (**606**), a **DAB(E)C** potential acceptor acting as a donor (**607**), and the recently disclosed acceptor **608** (K. Wright, C. Guerreiro, I. Laurent, F. Baleux, L. A. Mulard, *Org. Biomol. Chem.* **2004**, *2*, 1518-1527), bearing a masked aminoethyl spacer, as a precursor to the reducing end **D** residue (Figure 24). Although permanent blocking of OH-4<sub>D</sub> and OH-6<sub>D</sub> with an isopropylidene acetal may appear somewhat unusual, this choice was a key feature of the strategy. It was based on former observations in the methyl glycoside series, demonstrating that its use could overcome some of the known drawbacks of the corresponding benzylidene acetal (F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) J. Banoub, D. R. Bundle, *Can. J. Chem.* **1979**, *57*, 2091), including its poor solubility. In order to reduce the number of synthetic steps, it was found appropriate to access the **AB(E)C** donor and the **DAB(E)C** building block from a common key **AB(E)C** tetrasaccharide intermediate **609**. Most of all, the design of the pentasaccharide building block **607** was a key element to success. Indeed, a leading concept of the overall strategy was to limit the number of transformations at later stages in the syntheses. Concerning the choice of **607**, the reader's attention is thus drawn to (i) the permanent blocking of position 4<sub>D</sub> and 6<sub>D</sub> as an isopropylidene acetal, (ii) the introduction of a participating benzoyl group, resistant to Zemplén deacylation, at position 2<sub>A</sub>, (iii) the temporary protection of position 3<sub>D</sub> as an orthogonal acetate, (iv) the early introduction of the required 2<sub>D</sub> acetamido functionality and (v) the activation of the anomeric position as a trichloroacetimidate. Indeed, it should be outlined that the syntheses disclosed herein are based on the use of the trichloroacetimidate (TCA) chemistry, (R. R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21-123) and that known building blocks were used whenever possible.

*Synthesis of the tetrasaccharide building block 606 (Figure 25)*

Preparation of **606** was conveniently achieved from the previously described tetrasaccharide **609**, (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074) in a non optimized yield of 56%, according to a conventional protocol, namely selective removal of the anomeric allyl group and subsequent activation upon reaction of the resulting hemiacetal with trichloroacetonitrile in the presence of catalytic 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

*Synthesis of the pentasaccharide building block 607 (Figure 25):* Starting from **609**, we recently described the synthesis of the **DAB(E)C** building block **610** bearing a trichloroacetamide function at position 2<sub>D</sub>. This crucial intermediate could be obtained in high yield when running the condensation on a 5 g scale. It was used successfully as the donor in the synthesis of the **D'A'B'(E')C'DAB(E)C** decasaccharide, once converted to the corresponding trichloroacetimidate. (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074) However, for the present purpose we reasoned that conversion of the trichloroacetamide moiety into the required acetamide at an early stage in the synthesis was preferable. Thus, reductive free-radical dechlorination of **610** using Bu<sub>3</sub>SnH in the presence of catalytic AIBN allowed the conversion of the *N*-trichloroacetyl moiety into *N*-acetyl, to give the known **611** (68%), previously obtained according to an alternative and somewhat lower yielding strategy (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074). Controlled de-*O*-acetylation of **611** under Zemplén conditions gave the triol **612**, which was next converted to the corresponding alcohol **613** upon reaction with 2,2-dimethoxypropane (81% from **611**). Conventional acetylation at position 3<sub>D</sub> then gave the fully protected intermediate **614** (94%), the good overall yield of this three-step conversion (**611**→**614**, 76%) outlining its interest. The latter was transformed into the hemiacetal **615** (82%) following a two-step process including Iridium complex promoted isomerisation of the allyl moiety into the corresponding propen-1-yl (J. J. Oltvoort, C. A. A. van Boeckel, J. H. der Koning, J. van Boom, *Synthesis* **1981**, 305), and hydrolysis of the latter upon treatment with mercuric chloride, since it was originally demonstrated that labile isopropylidene groups were stable to such neutral conditions (R. Gigg, C. D. Warren, *J. Chem. Soc. C* **1968**, 1903). Subsequent reaction of **615** with trichloroacetonitrile in the presence of catalytic 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) cleanly gave the key building block **607** (85% from **614**).

*Synthesis of the aminoethyl decasaccharide 603 (Figure 26):* Previous glycosidation attempts in the series indicated that when run at low temperature or room temperature, reactions using the **D** acceptor **608** occasionally resulted in a somewhat poor yield of the condensation product. This was tentatively explained by the still rather low solubility of **608**. When using 1,2-dichloroethane (1,2-DCE) as the solvent, the condensation could be performed at higher temperature, which proved rewarding. Indeed, optimized coupling conditions of **607** and **608**, used in slight excess, relied on the concomitant use of a catalytic amount of triflic acid in the presence of 4Å molecular sieves as the promoter and 1,2-DCE as the solvent, while the condensation was performed at 75°C, according to a known protocole (F. Bélot, D. Rabuka, M. Fukuda, O. Hindsgaul, *Tetrahedron Lett.* **2002**, *43*, 7743) which had recently been adapted to the use of acceptor **608** in the *S. flexneri* series. The fully protected hexasaccharide **616** was isolated in a

satisfactory 76% yield. The resistance of the two isopropylidene acetals to the harsh acidic conditions of the glycosidation reaction is noteworthy. That the hemiacetal **615**, resulting from the hydrolysis of the excess donor could be recovered was of great advantage if one considers scaling up the process (not described). Resistance of isolated benzoyl groups to Zemplén transesterification has been reported (A. Liptak, Z. Szurmai, P. Nanasi, A. Neszmelyi, *Carbohydr. Res.* **1982**, *99* ; Z. Szurmai, J. Kerékgyarto, J. Harangi, A. Liptak, *Carbohydr. Res.* **1987**, *174*, 313 ; Z. Szurmai, A. Liptak, G. Snatzke, *Carbohydr. Res.* **1990**, *200*, 201). It was also observed previously in the series, upon attempted removal of a benzoyl group located at position 2<sub>C</sub>. Thus, as anticipated selective deacetylation at the 3-OH of the non reducing residue, gave the **D'AB(E)CD** acceptor **617** in a yield of 97%, which confirmed the orthogonality of the various protecting groups in use at this stage. Condensation of the latter and **606** was performed in 1,2-DCE using triflic acid as the promoter. One may note that although the condensation involves the construction of the C-D linkage, thus somewhat resembling the preparation of the hexasaccharide **616**, heating was not required and the glycosylation went smoothly at 10°C to give the fully protected decasaccharide **618** (82%). Acidic hydrolysis of the acetals gave the tetraol **619** (75%). Transesterification of the acyl groups was best performed by overnight heating of **619** in methanolic sodium methoxide. Final hydrogenolysis of the benzyl groups and concomitant conversion of the azido group into the corresponding amine gave the target **603** (71% from **619**). As observed earlier, (Q. Li, H. Li, Q.-H. Lou, B. Su, M.-S. Cai, Z.-J. Li, *Carbohydr. Res.* **2002**, *337*, 1929) the latter transformation was best performed under acidic conditions.

*Synthesis of the aminoethyl pentadecasaccharide 604 (Figure 27):* The rather convenient access to the building block **607** allowed the targeting of larger sequences. Thus, having the hexasaccharide acceptor **617** in hands, the two-step glycosylation/deacetylation process involving **607** was repeated. Analogously to the condensation step leading to the fully protected decasaccharide, condensation of **617** and the pentasaccharide donor **607** in the presence of triflic acid was run at a temperature below 5°C. Under such conditions, the fully protected undecasaccharide **621** was isolated in an excellent yield of 90%, outlining once more the compatibility of rather labile isopropylidene groups with the glycosylation conditions in use. Zemplén transesterification at the non reducing 3<sub>D</sub>-OH of the latter, resulting in the required acceptor **622** (91%), proved as efficient. Condensation of this key intermediate with the tetrasaccharide trichloroacetimidate donor **606** was performed according to the same protocole, using triflic acid as the promoter. The fully protected pentadecasaccharide **623** was isolated in a satisfactory yield of 82%. Conversion of **623** to the target **604** was performed by running the stepwise sequence described for the preparation of **603**. Acidic hydrolysis of the isopropylidene groups afforded the hexaol **624** (83%). Again, running the transesterification step at high temperature allowed to overcome the resistance of the

isolated 2C-benzoyl groups to methanolic transesterification. Lastly, conventional hydrogenolysis of the benzyl groups and concomitant reduction of the azide moiety allowed the smooth conversion of de-O-acylated intermediate into the pentadecasaccharide hapten **604** (65% from **624**). Interestingly, although the number of synthetic steps involved may be somewhat challenging, those are in average high yielding, making large amounts of **604** reachable.

#### *Synthesis of the target conjugates 601 and 602 (Figure 24)*

Chemoselective ligation of the carbohydrate B and peptide T epitopes was achieved through coupling of the carbohydrate haptens pre-functionalized with a thiol function and a maleimido group properly introduced at the C terminus of the T helper peptide, which allows specific and high-yielding modification of the former in the presence of other nucleophiles (G. T. Hermanson, *Bioconjugate techniques*, Academic Press, New York 1996). Based on reported data on the immunogenicity of various maleimide-derived coupling agents (J. M. Peeters, T. G. Hazendonk, E. C. Beuvery, G. I. Tesser, *J. Immunol Methods* 1989, 120, 133), 4-(N-maleimido)-n-butanoyl was selected as the linker. It was covalently linked to the side chain amino group of a lysine residue added to the C-terminus of the PADRE sequence (PADRE-Lys) according to an in-house process (K. Wright, C. Guerreiro, I. Laurent, F. Baleux, L. A. Mulard, *Org. Biomol. Chem.* 2004, 2, 1518) differing from that described previously by others (J. Alexander, A.-F. d. Guercio, A. Maewal, L. Qiao, J. Fikes, R. W. Chesnut, J. Paulson, D. R. Bundle, S. DeFrees, A. Sette *J. Immunol.* 2000, 164, 1625). Reaction of **603** and **604** with S-acetylthioglycolic acid pentafluorophenyl ester (SAMA-Pfp) resulted in the site-selective elongation of their aminoethyl spacer with a thioacetyl acetamido linker, yielding **620** (Figure 26) and **621** (Figure 27) in 61% and 63% yield, respectively. Derivatization could be monitored by RP-HPLC with detection at 215 nm and structure confirmation was based on MS and NMR analysis. Conjugation of the carbohydrate haptens to the maleimido activated PADRE-Lys (**605**) was run in phosphate buffer at pH 6.0 in the presence of hydroxylamine (H. F. Brugghe, H. A. M. Timmermans, L. M. A. van Unen, G. J. T. Hove, G. W. der Werken, J. T. Poolman, P. Hoogerhout, *Int. J. Peptide Protein Res.* 1994, 43, 166) and monitored by RP-HPLC. Lastly, RP-HPLC purification gave the target neoglycopeptides **601** and **602** as single products, whose identity was assessed by MS analysis, in yields of 44% and 67% respectively.

#### G. Synthesis of biotinylated analogues of oligosaccharide representative of fragments of the O-SP of *S. flexneri* 2a

The tri- (ECD), tetra- (B(E)CD), penta- (AB(E)CD), hexa (D'AB(E)CD), deca- ({AB(E)CD}<sub>2</sub>) and pentadecasaccharide ({AB(E)CD}<sub>3</sub>) were synthesized as their biotine conjugates **708-713**, respectively (Figure 28). Analogously to that used for the preparation of the corresponding glycopeptides, the synthetic strategy

relied on a chemoselective ligation step between a commercially available maleimide-activated biotine derivative **707** and the saccharides functionalized as thiols. The known thioacetates **701-703**, disclosed in our reports on the synthesis of the PADRE-conjugates (K. Wright, C. Guerreiro, I. Laurent, F. Baleux, L. A. Mulard, *Org. Biomol. Chem.* **2004**, 2, 1518), **704** (see part D, compound **413**), and **705-706** (see part F, compounds **620** and **625**, respectively) were used as precursors to the required thiols. Accordingly, conjugation of the carbohydrate haptens to the maleimido activated biotine (**707**) was run in phosphate buffer at pH 6.0 in presence of hydroxylamine (H. F. Brugghe, H. A. M. Timmermans, L. M. A. van Unen, G. J. T. Hove, G. W. der Werken, J. T. Poolman, P. Hoogerhout, *Int. J. Peptide Protein Res.* **1994**, 43, 166) and monitored by RP-HPLC. Lastly, RP-HPLC purification gave the target conjugates as single products, whose identity was assessed based on MS analysis.

#### **H. Synthesis of a *Shigella flexneri* 2a pentasaccharide-PADRE conjugate using an alternate conjugation chemistry**

We report herein on the synthesis of the (2-bromoethyl)carbonylaminoethyl glycoside of the pentasaccharide AB(E)CD (**802**) and on that of the corresponding fully synthetic conjugate (**801**) using the PADRE as a universal T-helper peptide (see section E for the background). The target **801** was obtained by chemoselective ligation of **802** to the side chain thiol group of a cysteine residue added at the C-terminus of the PADRE sequence (PADRE-Cys, **803**).

(3-Bromopropionyl) was selected as the linker, and incorporated using the succinimidyl intermediate **804**, itself prepared in one step from commercially available 3-bromopropionic acid (86%). Thus, reaction of **805** with **804** resulted in the site-selective elongation of the aminoethyl spacer via a 3-bromopropionyl linker. Derivatization could be monitored by RP-HPLC with detection at 215 nm. Under these conditions, the intermediate **802** was isolated in 69% yield. Its structure was confirmed based on MS and NMR analysis (not described). The PADRE-Cys sequence was assembled using standard Fmoc chemistry for solid-phase peptide synthesis (Chan, W. C.; White, P. D. *Fmoc solid phase peptide synthesis*; Oxford University Press: New York, 2000). Standard side chain protecting groups were used. Conjugation of the carbohydrate hapten **802** to the PADRE-Cys (**803**) was run in anhydrous DMF and monitored by RP-HPLC. Lastly, preparative RP-HPLC purification gave the target neoglycopeptide **801** (57%) as a single product, whose identity was assessed based on MS analysis.

#### **EXPERIMENTAL**

##### **Legend of figures :**

Figure 1 : Synthesis of the linear ECDAB-OMe pentasaccharide **101**

Figure 2 : Retrosynthetic analysis of pentasaccharide **102**



Figure 3 : Synthesis of the trisaccharide 125 (intermediate for the synthesis of the pentasaccharide 102

Figure 4 : Synthesis of the AB(E)CD pentasaccharide 102

Figure 5 : Representation of the orthoester 135

5 Figure 6 : Synthesis of the B(E)CD tetrasaccharide 103

Figure 7 : Pentasaccharides 201 (DAB(E)C), 202, 203

Figure 8 : Synthesis of compound 208

Figure 9 : Synthesis of compound 212

Figure 10 : Synthesis of the pentasaccharide 203

10 Figure 11 : Retrosynthetic analysis of the target decasaccharide D'A'B'(E')C'DAB(E)C 301

Figure 12 : Synthesis of the pentasaccharides 302, 303, 304

Figure 13 : Synthesis of the pentasaccharide 313

Figure 14 : Synthesis of the tetrasaccharides 338, 339, 340, 341

15 Figure 15 : Synthesis of the pentasaccharide 346

Figure 16 : Synthesis of the decasaccharide D'A'B'(E')C'DAB(E)C 301

Figure 17 : Retrosynthetic analysis of the target conjugate 401

Figure 18 : Synthesis of the hexasaccharide 402

Figure 19 : Retrosynthetic analysis of the target conjugates 501, 502, 503

20 Figure 20 : Synthesis of the aminoethyl ECD building block 518

Figure 21 : Synthesis of the aminoethyl tetrasaccharide 525

Figure 22 : Synthesis of the aminoethyl pentasaccharide 537

Figure 23 : Synthesis of the conjugates 501, 502, 503

Figure 24 : Retrosynthetic analysis of the target conjugates 601, 602

25 Figure 25 : Synthesis of the pentasaccharides 606 and 607

Figure 26 : Synthesis of the decasaccharide 620

Figure 27 : Synthesis of the pentadecasaccharide 625

Figure 28 : Synthesis of the conjugates 701 to 713

Figure 28bis: Synthesis of the conjugate 801.

30 Figure 29 illustrates the structure of the repeating units of the O-SP of *S. flexneri* serotype 2a.

- Figure 30 illustrates the protection conferred by immune serum specific for *S. flexneri* 2a LPS intranasally administered prior to i.n. challenge.

35 A. Serum IgG subclasses elicited in mice upon i.p. immunization with killed *S. flexneri* 2a bacteria. represents the mean value of the antibody titer (n=10 mice).

B. Protection assessed by reduction of lung-bacterial load in mice receiving anti-*S. flexneri* 2a LPS immune serum raised upon i.p. immunization, 1h prior to

i.n. challenge with a sublethal dose of *S. flexneri* 2a bacteria. a, b, c, correspond to immune sera exhibiting an anti-*S. flexneri* 2a LPS IgG antibody titer of 1/4,000, 1/16,000 and 1/64,000, respectively. Standard deviation is indicated (n=10 mice per group).

- Figure 31 illustrates the protection conferred by different subclasses of mIgG specific for *S. flexneri* 2a serotype determinants. A : mice receiving intranasally 20µg and 2µg of purified mIgG (F22, D15, A2, E4 or C1), respectively, 1h prior to i.n. challenge with a sublethal dose of *S. flexneri* 2a bacteria. Lung-bacterial load was expressed using arbitrary units with 100 corresponding to the bacterial count in lungs of control mice. Standard deviations are represented (n=10 mice per group ; 3 independent experiments). B : Histopathological study of mouse lungs. Upper row: control mice. Lower row: mice receiving mIgG. HE staining : a and d magnification x 40 ; b and e magnification x 100. Immunostaining using an anti-LPS antibody specific for *S. flexneri* serotype 2a : c and f magnification x100.

- Figure 32 illustrates the serotype-specific protection conferred by the anti-O-SP mIgGs. A : Mice were receiving i.n. 20µg of each of the purified mIgG, C20 and C1-7, 1h prior to i.n. challenge with a sublethal dose of *S. flexneri* serotype 2a (A) or serotype 5a (B) bacteria. Lung-bacterial load was expressed using arbitrary units with 100 corresponding to the bacterial count in lungs of control mice. Standard deviations are represented (n=10 mice per group; 3 independent experiments). B: Histopathological study of mouse lungs. a and b : mice receiving mIgGC20 specific for *S. flexneri* serotype 5a and challenged with *S. flexneri* serotype 2a and 5a, respectively. c and d : mice receiving mIgGC1-7 specific for *S. flexneri* 2a prior to challenge with *S. flexneri* serotype 2a and 5a, respectively. HE staining, magnification x 100.

- Figure 33 illustrates the protection conferred by mIgG specific for *S. flexneri* IpaB or IpaC invasins. Mice were receiving i.n. 20µg of each of the purified mIgG, H4, H16, J22, K24, and C20, 1h prior to i.n. challenge with a sublethal dose of *S. flexneri* serotype 5a. Lung-bacterial load was expressed using arbitrary units with 100 corresponding to the bacterial count in lungs of control mice. Standard deviations are represented (n=10 mice per group).

- Figure 34 illustrates the protection conferred by oligosaccharides-tetanus toxoid conjugates in the mouse model of pulmonary infection. For each mice tested, the bacteria load 24 hours after the challenge is indicated as a function of the anti-LPS 2a antibody titer before the challenge.

#### **I- Synthesis of oligosaccharides, polysaccharides and conjugates according to the invention**

**General Methods.** Melting points were determined in capillary tubes with an electrothermal apparatus and are uncorrected. Optical rotations were measured for CHCl<sub>3</sub> solutions at 25°C, except where indicated otherwise. TLC on precoated slides of Silica Ge

60 F<sub>254</sub> (Merck) was performed with solvent mixtures of appropriately adjusted polarity. Detection was effected when applicable, with UV light, and/or by charring with orcinol (3: mM) in 4N aq H<sub>2</sub>SO<sub>4</sub>. Preparative chromatography was performed by elution from column of Silica Gel 60 (particle size 0.040-0.063 mm). RP-HPLC (215 nm or 230 nm) used  
 5 Kromasil 5 µm C18 100Å 4.6x250 mm analytical column (1 mL.min<sup>-1</sup>). NMR spectra were recorded at 20°C on a Bruker Avance 400 spectrometer (400 MHz for <sup>1</sup>H, 100 MHz for <sup>13</sup>C) at 20°C. Unless indicated otherwise, NMR spectra were run for solutions in CDCl<sub>3</sub> using TMS (0.00 ppm for both <sup>1</sup>H and <sup>13</sup>C) as an external reference. Dioxane (67.4 ppm for <sup>13</sup>C) and trimethylsilyl-3-propionic acid sodium salt (0.00 ppm for <sup>1</sup>H) were used as external  
 10 references for solutions in D<sub>2</sub>O. Proton-signal assignments were made by first-order analysis of the spectra, as well as analysis of 2D <sup>1</sup>H-<sup>1</sup>H correlation maps (COSY) and selective TOCSY experiments. In the NMR spectra, of the two magnetically non-equivalent geminal protons at C-6, the one resonating at lower field is denoted H-6a and the one at higher field is denoted H-6b. The <sup>13</sup>C NMR assignments were supported by 2D <sup>13</sup>C-<sup>1</sup>H  
 15 correlations maps (HETCOR). Interchangeable assignments in the <sup>13</sup>C NMR spectra are marked with an asterisk in listing of signal assignments. Sugar residues in oligosaccharide are serially lettered according to the lettering of the repeating unit of the O-SP and identified by a subscript in listing of signal assignments. Low resolution mass spectra were obtained by either chemical ionisation (CI-MS) using NH<sub>3</sub> as the ionising gas, by  
 20 electrospray mass spectrometry (ES-MS), by fast atom bombardment mass spectrometry (FAB-MS) recorded in the positive-ion mode using dithioerythritol/dithio-L-threitol (4:1 Magic Bullet) as the matrix in the presence of NaI, and Xenon as the gas. HRMS were obtained by Matrix Assisted Laser Desorption Ionisation (MALDI).

#### Abréviations

25 TCA: trichloroacetimidate  
 EtOAc: Ethyl acetate  
 1,2-DCE: 1,2-dichloroethane  
 DCM: Dichloromethane  
 THF: Tetrahydrofuran  
 30 DMF: N,N-dimethyl formamide  
 rt: room temperature

#### A- Synthesis of the methyl glycosides of a tetra- and two pentasaccharide fragments of the O-specific polysaccharide of *Shigella flexner* serotype 2a :

35 Appropriate solvents for chromatography consisted of A, dichloromethane-methanol; B, cyclohexane-ethyl acetate, C, cyclohexane-acetone, D, water-acetonitrile, E, *iso*-propanol-ammonia-water; F, 0.01 M aq TFA-acetonitrile.

**Methyl (3,4-di-O-benzyl-2-O-chloroacetyl- $\alpha$ -L-rhamnopyranosyl) (1 $\rightarrow$ 2)-3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranoside (108).** Activated powered 4Å molecular sieves (200 mg) was added to a solution of alcohol (V. Pozsgay, J.-R. Brisson, H. J. Jennings, *Can. J. Chem.* **1987**, *65*, 2764-2769) **104** (60 mg, 167  $\mu$ mol) and trichloroacetimidate donor **120** (113 mg, 0.2 mmol) in dry Et<sub>2</sub>O (2 mL) and the solution was stirred at rt for 30 min then cooled to -40°C. TMSOTf (9  $\mu$ L, 50  $\mu$ mol) was added and the mixture was stirred for 1 h at -30°C, then for 2 h while the bath temperature was coming back to rt. TLC (solvent B, 4:1) showed the presence of less polar product than **104**. The mixture was neutralized by addition of Et<sub>3</sub>N, and filtered on a pad of Celite. Concentration of the filtrate and column chromatography of the residue (solvent B, 4:1) gave 86 mg of **108** as a colourless oil (67%).  $[\alpha]_D -13.6$  (*c* 1.0); <sup>1</sup>H NMR  $\delta$  7.42-7.32 (m, 20H, Ph), 5.64 (dd, 1H, J<sub>1,2</sub> = 1.9, J<sub>2,3</sub> = 3.2 Hz, H-2<sub>A</sub>), 5.07 (d, 1H, H-1<sub>A</sub>), 4.98-4.93 (m, 2H, OCH<sub>2</sub>), 4.83-4.6 (m, 6H, OCH<sub>2</sub>), 4.64 (bs, 1H, H-1<sub>B</sub>), 4.18 (d, 1H, J = 15.2 Hz, CH<sub>2</sub>Cl), 4.13 (d, 1H, OCH<sub>2</sub>Cl), 3.90 (dd, 1H, J<sub>3,4</sub> = 9.3 Hz, H-3<sub>B</sub>), 3.89 (m, 1H, partially overlapped, J<sub>5,6</sub> = 6.3 Hz, H-5<sub>A</sub>), 3.73 (dq, 1H, J<sub>4,5</sub> = 9.5, J<sub>5,6</sub> = 6.2 Hz, H-5<sub>B</sub>), 3.48 (pt, 1H, J<sub>3,4</sub> = 9.4 Hz, H-4<sub>B</sub>), 3.4 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 9.4 Hz, H-4<sub>A</sub>), 3.36 (s, 3H, OCH<sub>3</sub>), 1.37 (d, 3H, H-6<sub>A</sub>), 1.35 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR  $\delta$  165.5 (CO), 137.4-126.4 (Ph), 100.2 (C-1<sub>A</sub>), 99.2 (C-1<sub>B</sub>), 80.4, 80.3, 80.1 (2C, C-4<sub>A</sub>, 4<sub>B</sub>, 3<sub>B</sub>), 77.9 (C-3<sub>A</sub>), 75.8, 75.7 (2C, OCH<sub>2</sub>), 74.8 (C-2<sub>B</sub>), 72.6, 72.5 (2C, OCH<sub>2</sub>), 71.2 (C-2<sub>A</sub>), 68.7 (C-5<sub>A</sub>), 68.2 (C-5<sub>B</sub>), 55.0 (OCH<sub>3</sub>), 41.4 (CH<sub>2</sub>Cl), 18.4 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FABMS for C<sub>43</sub>H<sub>49</sub>ClNO<sub>10</sub> (M, 760.3) *m/z* 783.3 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>43</sub>H<sub>49</sub>ClNO<sub>10</sub>: C, 67.84; H, 6.49%. Found: C, 68.03; H, 7.02.

**Methyl (3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranoside (107).** Activated powered 4Å molecular sieves was added to a solution of alcohol **104** (322 mg, 0.90 mmol) and trichloroacetimidate donor (J. C. Castro-Palomino, M. H. Rensoli, V. Verez Bencomo, *J. Carbohydr. Chem.* **1996**, *15*, 137-146) **105** (573 mg, 1.08 mmol) in dry Et<sub>2</sub>O (9 mL) and the solution was stirred at rt for 30 min then cooled to -35°C. TMSOTf (48  $\mu$ L, 266  $\mu$ mol) was added and the mixture was stirred for 4 h, while the bath temperature was coming back to rt. TLC (solvent B, 23:2) showed that only little starting material remained and the mixture was neutralized by addition of Et<sub>3</sub>N, and filtered on a pad of Celite. Concentration of the filtrate and column chromatography of the residue (solvent B, 9:1) gave 647 mg of slightly contaminated **106**. The later (626 mg) was dissolved in a mixture of CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and MeOH (5 mL) and 1M methanolic sodium methoxide (300  $\mu$ L) was added. The mixture was stirred overnight, neutralized with Amberlite IR 120 (H<sup>+</sup>), filtered and concentrated. Chromatography of the residue (solvent G, 89:11) gave syrupy **107** (554 mg, 91% from **104**). Analytical data were as described.(V. Pozsgay, J.-R. Brisson, H. J. Jennings, *Can. J. Chem.* **1987**, *65*, 2764-2769)

**Methyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranoside (110).** A solution of disaccharide **107** (179 mg, 0.26 mmol) and trichloroacetimidate donor(J. C. Castro-Palomino, R. R. Schmidt, *Tetrahedron Lett.* **1995**, *36*, 5343-5346) **109** (436 mg, 0.60 mmol) in dry CH<sub>3</sub>CN (9 mL) was stirred at rt for 30 min in the presence of activated 4Å molecular sieves (1.2 g). Tin(II) trifluoromethanesulfonate [Sn(OTf)<sub>2</sub>] (75 mg, 180 μmol) was added and the mixture was stirred at rt for 4 h, then neutralized with Et<sub>3</sub>N. Filtration on a pad of Celite, concentration of the filtrate and column chromatography of the residue (solvent B, 87:13) gave **110** (324 mg) as a slightly contaminated white foam (72% as estimated from the <sup>1</sup>H NMR spectrum). An analytical sample had [ $\alpha$ ]<sub>D</sub> +23.3 (c 1.0); <sup>1</sup>H NMR  $\delta$  7.43-7.17 (m, 20H, Ph), 5.92 (d, 1H, J = 9.2, J = 10.5 Hz, H-3<sub>D</sub>), 5.24 (d, 1H, J<sub>1,2</sub> = 8.4 Hz, H-1<sub>D</sub>), 5.14 (dd, 1H, J = 9.7, J = 9.4 Hz, H-4<sub>D</sub>), 5.00 (bs, 1H, H-1<sub>A</sub>), 4.79 (d, 1H, J = 10.8 Hz, OCH<sub>2</sub>), 4.65 (s, 2H, OCH<sub>2</sub>), 4.55 (d, 1H, J = 11.2 Hz, OCH<sub>2</sub>), 4.53 (bs, 1H, H-1<sub>B</sub>), 4.46-4.36 (m, 3H, H-2<sub>D</sub>, OCH<sub>2</sub>), 4.28 (d, 1H, J = 12.4 Hz, OCH<sub>2</sub>), 4.26 (d, 1H, J = 10.6 Hz, OCH<sub>2</sub>), 4.06 (dd, 1H, J<sub>6a,6b</sub> = 12.5, J<sub>5,6a</sub> = 6.8 Hz, H-6a<sub>D</sub>), 3.91 (bs, 1H, H-2<sub>B</sub>), 3.85-3.69 (m, 5H, H-2<sub>A</sub>, 3<sub>B</sub>, 3<sub>A</sub>, 6b<sub>D</sub>, 5<sub>A</sub>\*), 3.59 (dq, 1H, J<sub>4,5</sub> = 9.4, J<sub>5,6</sub> = 6.2 Hz, H-5<sub>B</sub>\*), 3.40 (m, 1H, H-5<sub>D</sub>), 3.27 (s, 3H, OCH<sub>3</sub>), 3.18 (m, 2H, H-4<sub>A</sub>, 4<sub>B</sub>), 2.03, 2.01, 1.94 (3s, 9H, C(O)CH<sub>3</sub>), 1.27, 1.25 (2d, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR  $\delta$  170.5, 170.4, 170.3, 163.8, 162.6 (5C, CO), 140.3-128.0 (Ph), 101.1 (C-1<sub>A</sub>), 100.0 (C-1<sub>D</sub>), 99.8 (C-1<sub>B</sub>), 80.7 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 79.7 (C-2<sub>A</sub>), 78.9 (C-3<sub>B</sub>), 78.1 (C-3<sub>A</sub>), 76.2 (C-2<sub>B</sub>), 75.3, 75.2, 72.7, 71.4 (4C, OCH<sub>2</sub>), 71.3 (C-5<sub>D</sub>), 70.1 (C-3<sub>D</sub>), 68.5 (C-5<sub>A</sub>\*), 68.4 (C-4<sub>D</sub>), 67.4 (C-5<sub>B</sub>\*), 61.3 (C-6<sub>D</sub>), 55.4 (C-2<sub>D</sub>), 54.6 (OCH<sub>3</sub>), 20.7, 20.6 (3C, C(O)CH<sub>3</sub>), 18.0, 17.7 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FABMS for C<sub>61</sub>H<sub>63</sub>Cl<sub>4</sub>NO<sub>18</sub> (M, 1237.3) *m/z* 1259.9 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>61</sub>H<sub>63</sub>Cl<sub>4</sub>NO<sub>18</sub>·H<sub>2</sub>O: C, 58.24; H, 5.21; N, 1.11%. Found: C, 58.21; H, 4.91; N, 1.01%.

**Methyl (2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranoside (111).** A solution of disaccharide **107** (179 mg, 0.26 mmol) and trichloroacetimidate donor **109** (436 mg, 0.60 mmol) in dry CH<sub>3</sub>CN (9 mL) was stirred at rt for 30 min in the presence of activated 4Å molecular sieves (1.2 g). Tin(II) trifluoromethanesulfonate [Sn(OTf)<sub>2</sub>] (75 mg, 180 μmol) was added and the mixture was stirred at rt for 4 h, then neutralized with Et<sub>3</sub>N. Filtration on a pad of Celite, concentration of the filtrate and column chromatography of the residue (solvent B, 87:13) gave **110** (324 mg) as a slightly contaminated product. The latter was solubilized in dry ethanol (13 mL) and diethylamine (200 μL, 3.0 mmol) was added and the mixture was stirred overnight at 60°C. The mixture was cooled to rt and acetic anhydride (1.0 mL, 10.6 mmol) was added and the mixture was stirred at this temperature for 2 h. The suspension was filtered and volatiles were evaporated and coevaporated repeatedly with toluene and cyclohexane. The crude residue

was taken up in a minimum of CH<sub>2</sub>Cl<sub>2</sub> and MeOH (10 mL). 1N methanolic sodium methoxide was added until the pH was 10 and the solution was stirred overnight at rt, neutralized with IR 120 (H<sup>+</sup>), filtered and concentrated. Chromatography of the residue (solvent A, 24:1) gave foamy **111** (135 mg, 51% from **107**). [ $\alpha$ ]<sub>D</sub> -15.0 (*c* 1.0); <sup>1</sup>H NMR  $\delta$  7.44-7.28 (m, 20H, Ph), 8.88 (bs, 1H, NH<sub>D</sub>), 5.28 (bs, 1H, H-1<sub>A</sub>), 4.93-4.61 (m, 8H, OCH<sub>2</sub>), 4.59 (s, 1H, J<sub>1,2</sub> = 1.3 Hz, H-1<sub>B</sub>), 4.41 (d, 1H, J<sub>1,2</sub> = 8.3 Hz, H-1<sub>D</sub>), 4.06 (m, 2H, H-2<sub>A</sub>, 2<sub>B</sub>), 4.00 (dd, 1H, J<sub>2,3</sub> = 3.3, J<sub>3,4</sub> = 9.4 Hz, H-3<sub>A</sub>), 3.86 (dd, 1H, J<sub>2,3</sub> = 2.9, J<sub>3,4</sub> = 9.4 Hz, H-3<sub>B</sub>), 3.79 (dq, 1H, J<sub>4,5</sub> = 9.4, J<sub>5,6</sub> = 6.2 Hz, H-5<sub>A</sub>\*), 3.67 (m, 2H, H-5<sub>B</sub>\*, 6a<sub>D</sub>), 3.51 (m, 1H, H-2<sub>D</sub>), 3.49-3.38 (m, 6H, H-6b<sub>D</sub>, 4<sub>D</sub>, 3<sub>D</sub>, 4<sub>B</sub>, 4<sub>A</sub>), 3.31 (s, 3H, OCH<sub>3</sub>), 3.29 (m, 1H, H-5<sub>D</sub>), 1.55 (s, 3H, C(O)CH<sub>3</sub>), 1.35 (d, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR  $\delta$  173.6 (CO), 138.5-127.6 (Ph), 103.2 (C-1<sub>D</sub>), 100.2 (C-1<sub>A</sub>), 99.9 (C-1<sub>B</sub>), 81.3, 80.7 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 79.9 (2C, C-3<sub>A</sub>, 3<sub>B</sub>), 79.0 (C-2<sub>A</sub>), 77.2 (C-3<sub>D</sub>), 75.8 (C-5<sub>D</sub>), 75.7, 75.2, 74.6 (3C, OCH<sub>2</sub>), 73.4 (C-2<sub>B</sub>), 72.3 (OCH<sub>2</sub>), 71.8 (C-4<sub>D</sub>), 68.2, 67.7 (C-5<sub>A</sub>, 5<sub>B</sub>), 62.5 (C-6<sub>D</sub>), 58.9 (C-2<sub>D</sub>), 54.6 (OCH<sub>3</sub>), 22.3 (C(O)CH<sub>3</sub>), 17.9, 17.7 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FABMS for C<sub>49</sub>H<sub>61</sub>NO<sub>14</sub> (M, 887.44) *m/z* 910.1 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>49</sub>H<sub>61</sub>NO<sub>14</sub>·H<sub>2</sub>O: C, 64.96; H, 7.01; N, 1.55%. Found: C, 65.19; H, 6.83; N, 1.51%.

**Methyl (2-acetamido-2-deoxy-4,6-O-isopropylidene- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranoside (112).** 2,2-dimethoxypropane (4.9 mL, 39.8 mmol) and *para*-toluenesulfonic acid (18 mg, 95  $\mu$ mol) were added to a solution of the triol **111** (964 mg, 1.09 mmol) in acetone (3 mL) and the mixture was stirred at rt for 1h. Et<sub>3</sub>N was added, and volatiles were evaporated. Column chromatography of the residue (solvent A, 99:1) gave the acceptor **112** as a white solid (969 mg, 96%) which could be crystallized from AcOEt:iPr<sub>2</sub>O; mp 164-165°C [ $\alpha$ ]<sub>D</sub> -25.9 (*c* 1.0); <sup>1</sup>H NMR  $\delta$  7.45-7.31 (m, 20H, Ph), 6.98 (d, 1H, J<sub>NH,2</sub> = 2.4 Hz, NH), 6.37 (bs, 1H, OH), 5.07 (d, 1H, J<sub>1,2</sub> = 1.9 Hz, H-1<sub>A</sub>), 4.90 (d, 1H, J = 10.8 Hz, OCH<sub>2</sub>), 4.85 (d, 1H, J = 10.1 Hz, OCH<sub>2</sub>), 4.84 (d, 1H, J = 10.8 Hz, OCH<sub>2</sub>), 4.76 (d, 1H, OCH<sub>2</sub>), 4.69 (d, 1H, OCH<sub>2</sub>), 4.68 (s, 2H, OCH<sub>2</sub>), 4.65 (d, 1H, OCH<sub>2</sub>), 4.61 (d, 1H, J<sub>1,2</sub> = 1.6 Hz, H-1<sub>B</sub>), 4.48 (d, 1H, J<sub>1,2</sub> = 8.3 Hz, H-1<sub>D</sub>), 4.09 (dd, 1H, H-2<sub>A</sub>), 4.01 (dd, 1H, J<sub>2,3</sub> = 3.2, J<sub>3,4</sub> = 9.4 Hz, H-3<sub>A</sub>), 3.91 (dd, 1H, H-2<sub>B</sub>), 3.89-3.84 (m, 2H, J<sub>5,6</sub> = 6.3, J<sub>4,5</sub> = 9.4, J<sub>2,3</sub> = 3.3, J<sub>3,4</sub> = 9.4 Hz, H-5<sub>A</sub>, 3<sub>B</sub>), 3.68 (dq, partially overlapped, J<sub>5,6</sub> = 6.2, J<sub>4,5</sub> = 9.5 Hz, H-5<sub>B</sub>), 3.66-3.58 (m, 5H, H-6a<sub>D</sub>, 6b<sub>D</sub>, 2<sub>D</sub>, 3<sub>D</sub>, 4<sub>D</sub>), 3.44 (pt, 1H, H-4<sub>A</sub>), 3.41 (pt, 1H, H-4<sub>B</sub>), 3.32 (s, 3H, OCH<sub>3</sub>), 3.16 (m, 1H, H-5<sub>D</sub>), 1.60 (s, 3H, C(O)CH<sub>3</sub>), 1.54, 1.48 (2s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 1.35 (d, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR  $\delta$  173.9 (CO), 138.8-128.0 (Ph), 103.7 (C-1<sub>D</sub>), 101.3 (C-1<sub>A</sub>), 100.3 (C(CH<sub>3</sub>)<sub>2</sub>), 100.2 (C-1<sub>B</sub>), 81.9 (C-4<sub>A</sub>), 80.8 (C-4<sub>B</sub>), 80.5 (C-3<sub>A</sub>), 79.7 (C-3<sub>B</sub>), 79.4 (C-2<sub>A</sub>), 76.2 (OCH<sub>2</sub>), 76.0 (C-2<sub>B</sub>), 75.6, 75.1 (2C, OCH<sub>2</sub>), 74.7 (C-4<sub>D</sub>), 74.4 (C-3<sub>D</sub>), 72.6 (OCH<sub>2</sub>), 68.6 (C-5<sub>A</sub>), 68.0, 67.9 (2C, C-5<sub>B</sub>, 5<sub>D</sub>), 62.2 (C-6<sub>D</sub>), 60.6 (C-2<sub>D</sub>), 55.1 (OCH<sub>3</sub>), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 22.7 (C(O)CH<sub>3</sub>), 19.4 (C(CH<sub>3</sub>)<sub>2</sub>), 18.5, 18.2 (2C, C-

6<sub>A</sub>, 6<sub>B</sub>). FABMS for C<sub>52</sub>H<sub>65</sub>NO<sub>14</sub> (M, 927.44) *m/z* 950.1 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>52</sub>H<sub>65</sub>NO<sub>14</sub>: C, 67.30; H, 7.06; N, 1.51%. Found: C, 67.12; H, 6.98; N, 1.44%.

**Methyl (2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3-di-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranoside (115).** Activated powdered 4Å molecular sieves were added to a solution of the trisaccharide acceptor **112** (202 mg, 0.22 mmol) and the disaccharide donor **114** (263 mg, 0.25 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and the suspension was stirred for 30 min at -15°C. TfOH (7  $\mu$ L, 34  $\mu$ mol) was added and the mixture was stirred for 2 h while the bath temperature was slowly coming back to 10°C. TLC (solvent D, 49:1) showed that no **112** remained. Et<sub>3</sub>N was added and after 30 min, the suspension was filtered through a pad of Celite. Concentration of the filtrate and chromatography of the residue (solvent B, 9:1  $\rightarrow$  17:5) gave the fully protected pentasaccharide **115** (330 mg, 84%) as a white foam; [ $\alpha$ ]<sub>D</sub> +63.3 (c 1.0); <sup>1</sup>H NMR  $\delta$  8.07-6.96 (m, 50H, Ph), 5.82 (d, 1H, J<sub>NH,2</sub> = 7.4 Hz, NH), 5.63 (dd, 1H, J<sub>2,3</sub> = 3.5, J<sub>3,4</sub> = 9.5 Hz, H-3<sub>C</sub>), 5.43 (dd, 1H, J<sub>1,2</sub> = 1.6 Hz, H-2<sub>C</sub>), 5.09 (bs, 1H, H-1<sub>A</sub>), 5.02 (d, 1H, J<sub>1,2</sub> = 3.4 Hz, H-1<sub>E</sub>), 4.99 (d, 1H, J<sub>1,2</sub> = 8.3 Hz, H-1<sub>D</sub>), 4.95 (d, 1H, J<sub>1,2</sub> = 1.1 Hz, H-1<sub>C</sub>), 4.94-4.63 (m, 13H, OCH<sub>2</sub>), 4.63 (s, 1H, H-1<sub>B</sub>), 4.37 (d, 1H, J = 11.0 Hz, OCH<sub>2</sub>), 4.29 (dq, 1H, J<sub>4,5</sub> = 9.5, J<sub>5,6</sub> = 6.2 Hz, H-5<sub>C</sub>), 4.25 (d, 1H, J = 9.5 Hz, OCH<sub>2</sub>), 4.23 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 9.5 Hz, H-3<sub>D</sub>), 4.01 (m, 1H, H-2<sub>A</sub>), 3.97-3.86 (m, 5H, H-3<sub>A</sub>, 2<sub>B</sub>, 3<sub>E</sub>, 4<sub>C</sub>, OCH<sub>2</sub>), 3.82 (m, 1H, H-3<sub>B</sub>, 5<sub>A</sub>), 3.71-3.57 (m, 7H, H-5<sub>D</sub>, 4<sub>E</sub>, 5<sub>B</sub>, 4<sub>D</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>), 3.54-3.41 (m, 3H, H-2<sub>E</sub>, 4<sub>A</sub>, 2<sub>D</sub>), 3.38-3.31 (m, 2H, H-4<sub>B</sub>, 6<sub>aE</sub>), 3.31 (s, 3H, OCH<sub>3</sub>), 3.17 (m, 1H, H-5<sub>D</sub>), 3.08 (d, 1H, J<sub>6a,6b</sub> = 10.1 Hz, H-6<sub>bE</sub>), 1.84 (s, 3H, C(O)CH<sub>3</sub>), 1.46 (s, 3H, C(CH<sub>3</sub>)<sub>2</sub>), 1.45 (d, 3H, J<sub>5,6</sub> = 5.9 Hz, H-6<sub>C</sub>), 1.35 (m, 6H, J<sub>5,6</sub> = 5.9 Hz, H-6<sub>A</sub>, C(CH<sub>3</sub>)<sub>2</sub>), 1.31 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>B</sub>); <sup>13</sup>C NMR  $\delta$  171.7, 165.9, 165.8 (3C, CO), 138.9-127.9 (Ph), 102.3 (C-1<sub>D</sub>, J = 167 Hz), 101.5 (C-1<sub>A</sub>, J = 170 Hz), 100.3 (C-1<sub>B</sub>, J = 170 Hz), 99.8 (C(CH<sub>3</sub>)<sub>2</sub>), 99.6 (C-1<sub>E</sub>, J = 172 Hz), 98.2 (C-1<sub>C</sub>, J = 172 Hz), 82.0 (C-3<sub>E</sub>), 81.2, 80.9, 80.7 (3C, C-4<sub>A</sub>, 4<sub>B</sub>, 2<sub>E</sub>), 80.0, 79.7, 79.3 (3C, C-3<sub>B</sub>, 3<sub>A</sub>, 4<sub>C</sub>), 78.1, 77.8, 77.4 (3C, C-2<sub>A</sub>, 4<sub>E</sub>, 3<sub>D</sub>), 75.9, 75.8, 75.6 (3C, OCH<sub>2</sub>), 75.5 (C-2<sub>B</sub>), 75.0, 74.4, 73.7 (3C, OCH<sub>2</sub>), 73.2 (2C, C-4<sub>D</sub>, OCH<sub>2</sub>), 72.2 (OCH<sub>2</sub>), 71.7, 71.6 (3C, C-2<sub>C</sub>, 3<sub>C</sub>, 5<sub>E</sub>), 68.8 (C-5<sub>B</sub>), 68.0 (C-6<sub>E</sub>), 68.0 (2C, C-5<sub>A</sub>, 5<sub>B</sub>), 67.6 (C-5<sub>D</sub>), 62.5 (C-6<sub>D</sub>), 58.9 (C-2<sub>D</sub>), 55.0 (OCH<sub>3</sub>), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 23.8 (C(O)CH<sub>3</sub>), 19.8 (C(CH<sub>3</sub>)<sub>2</sub>), 18.6 (C-6<sub>C</sub>), 18.5 (C-6<sub>A</sub>), 18.3 (C-6<sub>B</sub>). FAB-MS for C<sub>106</sub>H<sub>117</sub>NO<sub>25</sub> (M, 1803.79) *m/z* 1826.4 [M+H]<sup>+</sup>. Anal. Calcd for C<sub>106</sub>H<sub>117</sub>NO<sub>25</sub>·H<sub>2</sub>O: C, 69.83; H, 6.58; N, 0.77%. Found: C, 69.86; H, 6.33; N, 0.71%.

**Methyl (2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2,3-di-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranoside (116).** 90% aq TFA (750  $\mu$ L) was added at 0°C to a solution of the fully protected **115** (588 mg, 326  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (6.7 mL) and the mixture was stirred at

this temperature for 1 h. TLC (solvent *B*, 1.5:1) showed that no **115** remained. Volatiles were evaporated by repeated addition of toluene. Chromatography of the residue (solvent *B*, 4:1 → 1:1) gave **116** (544 mg, 95%) as a white foam;  $[\alpha]_D^{25} +58.8$  (*c* 1.0);  $^1\text{H}$  NMR  $\delta$  8.06-7.06 (m, 50H, Ph), 5.82 (d, 1H,  $J_{\text{NH},2} = 7.1$  Hz, NH), 5.65 (dd, 1H,  $J_{2,3} = 3.8$ ,  $J_{3,4} = 9.0$  Hz, H-3<sub>C</sub>), 5.53 (m, 1H, H-2<sub>C</sub>), 5.34 (bs, 1H, H-1<sub>A</sub>), 5.04 (d, 1H,  $J_{1,2} = 8.3$  Hz, H-1<sub>D</sub>), 5.00 (m, 2H, H-1<sub>C</sub>, 1<sub>E</sub>), 4.97-4.63 (m, 13H, OCH<sub>2</sub>), 4.48 (bs, 1H, H-1<sub>B</sub>), 4.40 (d, 1H,  $J = 8.4$  Hz, OCH<sub>2</sub>), 4.29 (d, 1H,  $J = 8.0$  Hz, OCH<sub>2</sub>), 4.28-4.21 (m, 2H, H-3<sub>D</sub>, 5<sub>C</sub>), 4.10 (m, 1H, H-2<sub>B</sub>), 4.04 (m, 1H, H-2<sub>A</sub>), 3.99 (d, 1H, OCH<sub>2</sub>), 3.95-3.89 (m, 3H, H-3<sub>A</sub>, 3<sub>E</sub>, 4<sub>C</sub>), 3.87 (dd, 1H,  $J_{2,3} = 2.7$ ,  $J_{3,4} = 9.7$  Hz, H-3<sub>B</sub>), 3.81-3.64 (m, 5H, H-5<sub>E</sub>, 5<sub>A</sub>, 6<sub>D</sub>, 4<sub>E</sub>, 5<sub>B</sub>), 3.54 (dd, 1H,  $J_{1,2} = 3.2$ ,  $J_{2,3} = 9.7$  Hz, H-2<sub>E</sub>), 3.51 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.5$  Hz, H-4<sub>A</sub>), 3.45-3.37 (m, 4H, H-4<sub>B</sub>, 4<sub>D</sub>, 6<sub>A</sub>, 2<sub>D</sub>), 3.33 (m, 5H, H-5<sub>D</sub>, 6<sub>B</sub>, OCH<sub>3</sub>), 3.12 (d, 1H,  $J_{6a,6b} = 10.6$  Hz, H-6<sub>b</sub>), 2.28 (bs, 1H, OH), 1.97 (bs, 1H, OH), 1.84 (s, 3H, C(O)CH<sub>3</sub>), 1.54 (d, 3H,  $J_{5,6} = 6.1$  Hz, H-6<sub>C</sub>), 1.37 (m, 6H, H-6<sub>B</sub>, 6<sub>A</sub>);  $^{13}\text{C}$  NMR  $\delta$  171.5, 165.8, 165.6 (3C, CO), 138.8-127.9 (Ph), 101.6 (C-1<sub>D</sub>), 100.8 (C-1<sub>A</sub>), 100.5 (C-1<sub>B</sub>), 100.1 (C-1<sub>E</sub>\*), 99.9 (C-1<sub>C</sub>\*), 84.9 (C-3<sub>D</sub>), 82.1 (C-3<sub>E</sub>), 80.9, 80.7, 80.6, 80.5 (4C, C-4<sub>B</sub>, 3<sub>B</sub>, 4<sub>A</sub>, 2<sub>E</sub>), 79.7 (C-4<sub>C</sub>), 79.3 (C-3<sub>A</sub>), 77.8 (2C, C-2<sub>A</sub>, 4<sub>E</sub>), 76.0, 75.9 (2C, OCH<sub>2</sub>), 75.8 (C-5<sub>D</sub>), 75.6, 75.1, 74.6, 73.7, 73.1 (5C, OCH<sub>2</sub>), 72.8 (C-2<sub>B</sub>), 72.6 (OCH<sub>2</sub>), 71.8 (C-5<sub>E</sub>), 71.6 (C-4<sub>D</sub>), 71.3 (C-3<sub>C</sub>), 71.1 (C-2<sub>C</sub>), 69.4 (C-5<sub>C</sub>), 68.8 (C-5<sub>A</sub>), 68.3 (C-5<sub>B</sub>), 68.1 (C-6<sub>E</sub>), 63.0 (C-6<sub>D</sub>), 57.6 (C-2<sub>D</sub>), 55.0 (OCH<sub>3</sub>), 23.8 (C(O)CH<sub>3</sub>), 18.8 (C-6<sub>C</sub>), 18.6, 18.5 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>103</sub>H<sub>113</sub>NO<sub>25</sub> (M, 1763.76) *m/z* 1786.2 [M+H]<sup>+</sup>. Anal. Calcd for C<sub>103</sub>H<sub>113</sub>NO<sub>25</sub>·2 H<sub>2</sub>O: C, 68.69; H, 6.55; N, 0.78%. Found: C, 68.74; H, 6.45; N, 0.65%.

**Methyl (2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1→4)- $\alpha$ -L-rhamnopyranosyl-(1→3)-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranoside (**117**).**

1M Methanolic sodium methoxide was added to a solution of **116** (277 mg, 157  $\mu\text{mol}$ ) in a 1:1 mixture of CH<sub>2</sub>Cl<sub>2</sub> and MeOH (6 mL) until the pH was 10. The mixture was stirred overnight at rt and neutralized with Amberlite IR-120 (H<sup>+</sup>). The crude material was chromatographed (solvent *A*, 49:1) to give **117** (211 mg, 86%) as a white foam;  $[\alpha]_D^{25} +23.8$  (*c* 1.0);  $^1\text{H}$  NMR  $\delta$  7.33-7.16 (m, 40H, Ph), 5.34 (d, 1H,  $J_{\text{NH},2} = 7.6$  Hz, NH), 5.18 (bs, 1H, H-1<sub>A</sub>), 4.79 (d, partially overlapped, 1H, H-1<sub>E</sub>), 4.67 (bs, 1H, H-1<sub>C</sub>), 4.50 (d, partially overlapped, 1H, H-1<sub>D</sub>), 4.49 (bs, 1H, H-1<sub>B</sub>), 4.88-4.33 (m, 16H, OCH<sub>2</sub>), 3.98-3.81 (m, 6H, H-2<sub>A</sub>, 2<sub>B</sub>, 5<sub>E</sub>, 3<sub>A</sub>, 3<sub>E</sub>, 5<sub>B</sub>\*), 3.77-3.70 (m, 3H, H-3<sub>B</sub>, 2<sub>C</sub>, 5<sub>C</sub>\*), 3.65 (dq, 1H,  $J_{4,5} = 9.4$ ,  $J_{5,6} = 6.2$  Hz, H-5<sub>A</sub>\*), 3.62-3.51 (m, 4H, H-2<sub>D</sub>, 6<sub>D</sub>, 6<sub>A</sub>, 6<sub>B</sub>), 3.48-3.27 (m, 7H, H-2<sub>E</sub>, 4<sub>E</sub>, 3<sub>D</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 3<sub>C</sub>, 4<sub>C</sub>), 3.23-3.12 (m, 6H, H-4<sub>D</sub>, 6<sub>B</sub>, 5<sub>D</sub>, OCH<sub>3</sub>), 2.76 (bs, 1H, OH), 1.72 (bs, 3H, OH), 1.65 (s, 3H, NHAc), 1.32, 1.25 (2d, 9H, H-6<sub>C</sub>, 6<sub>B</sub>, 6<sub>A</sub>);  $^{13}\text{C}$  NMR  $\delta$  170.6 (CO), 138.5-128.0 (Ph), 103.0 (C-1<sub>D</sub>), 101.8 (C-1<sub>C</sub>), 100.7 (C-1<sub>A</sub>), 100.4 (C-1<sub>B</sub>), 99.6 (C-1<sub>E</sub>), 87.3 (C-3<sub>D</sub>), 85. (C-4<sub>C</sub>\*), 82.0 (C-3<sub>E</sub>), 81.2, 80.7, 80.5, 80.2, 79.7, 78.1, 77.9 (7C, C-2<sub>B</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 2<sub>E</sub>, 4<sub>E</sub>), 76.2 (C-5<sub>D</sub>), 76.1, 75.9, 75.6, 75.4, 74.0, 73.9, 73.6 (7C, OCH<sub>2</sub>), 73.0 (C-2<sub>A</sub>),



72.8 (OCH<sub>2</sub>), 71.7, 71.2, 71.1, 69.8 (4C, C-4<sub>D</sub>, 5E, 2C, 3C), 68.8, 68.2 (3C, C-5<sub>A</sub>, 5B, 5C), 63.1 (C-6<sub>D</sub>), 55.6 (C-2<sub>D</sub>), 55.0 (OCH<sub>3</sub>), 23.7 (C(O)CH<sub>3</sub>), 18.6, 18.3, 18.1 (3C, C-6<sub>A</sub>, 6B, 6C). FAB-MS for C<sub>89</sub>H<sub>105</sub>NO<sub>23</sub> (M, 1555.71) *m/z* 1578.2 [M+H]<sup>+</sup>. Anal. Calcd for C<sub>89</sub>H<sub>105</sub>NO<sub>23</sub>: C, 68.66; H, 6.80; N, 0.90%. Found: C, 68.41; H, 6.78; N, 0.61%.

5                    **Methyl α-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside (101).** The benzylated tetrasaccharide 117 (352 mg, 226 μmol) was dissolved in a mixture of ethanol (14 mL) and AcOH (1 mL), treated with 10% Pd-C catalyst (200 mg), and the suspension was stirred for 5 days at rt. TLC (solvent A, 1:1) showed that the starting material had been transformed into a more polar product. The suspension was filtered on a pad of Celite. The filtrate was concentrated and coevaporated repeatedly with cyclohexane. Reverse phase chromatography of the residue (solvent D, 100:0 → 49:1), followed by freeze-drying, gave the target tetrasaccharide 101 as an amorphous powder (153 mg, 81%). RP-HPLC gave a single product eluting at Rt: 15.21 min (solvent F, 1:0 → 80:20 over 20 min); [α]<sub>D</sub> -3.2 (c 1.0, methanol); <sup>1</sup>H NMR (D<sub>2</sub>O) δ 5.08 (d, 1H, J<sub>1,2</sub> = 1.2 Hz, H-1<sub>A</sub>), 4.97 (d, 1H, J<sub>1,2</sub> = 3.9 Hz, H-1<sub>E</sub>), 4.79 (d, 1H, J<sub>1,2</sub> = 1.3 Hz, H-1<sub>C</sub>), 4.69 (m, 2H, H-1<sub>B</sub>, 1<sub>D</sub>), 4.07 (dd, 1H, J<sub>2,3</sub> = 3.3 Hz, H-2<sub>A</sub>), 4.02 (dq, 1H, J<sub>4,5</sub> = 9.3, J<sub>5,6</sub> = 6.2 Hz, H-5<sub>C</sub>), 3.93 (m, 1H, H-5<sub>E</sub>), 3.86 (m, 2H, H-2<sub>B</sub>, 3<sub>A</sub>), 3.82-3.73 (m, 7H, H-3<sub>C</sub>, 2<sub>D</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 3<sub>B</sub>, 2<sub>C</sub>, 6a<sub>D</sub>), 3.70-3.59 (m, 4H, H-5<sub>A</sub>, 3<sub>E</sub>, 6b<sub>D</sub>, 5<sub>B</sub>), 3.56 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 9.4 Hz, H-3<sub>D</sub>), 3.49 (dd, 1H, J<sub>2,3</sub> = 9.6 Hz, H-2<sub>E</sub>), 3.46-3.38 (m, 5H, H-4<sub>C</sub>, 4<sub>B</sub>, 4<sub>D</sub>, 5<sub>D</sub>, 4<sub>E</sub>), 3.32 (s, 3H, OCH<sub>3</sub>), 3.24 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 9.6 Hz, H-4<sub>A</sub>), 2.00 (s, 3H, C(O)CH<sub>3</sub>), 1.25 (d, 3H, partially overlapped, H-6<sub>C</sub>), 1.23 (d, 3H, partially overlapped, H-6<sub>B</sub>), 1.18 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>A</sub>); <sup>13</sup>C NMR (D<sub>2</sub>O) δ 175.0 (CO), 102.3 (C-1<sub>D</sub>, J = 162 Hz), 101.5 (C-1<sub>C</sub>, J = 170 Hz), 101.3 (C-1<sub>A</sub>, J = 173 Hz), 100.0 (C-1<sub>E</sub>, J = 170 Hz), 99.9 (C-1<sub>B</sub>, J = 172 Hz), 81.9 (C-3<sub>D</sub>), 81.4 (C-4<sub>C</sub>), 79.2 (C-2<sub>A</sub>), 79.0 (C-2<sub>B</sub>), 76.2, 73.1, 72.6, 72.2, 72.0, 71.4, 70.4, 70.0, 69.8, 69.7, 69.6, 69.3, 68.9, 68.7 (14C, 3<sub>A</sub>, 4<sub>A</sub>, 5<sub>A</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 5<sub>B</sub>, 2<sub>C</sub>, 3<sub>C</sub>, 4<sub>D</sub>, 5<sub>D</sub>, 2<sub>E</sub>, 3<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>), 68.4 (C-5<sub>C</sub>), 60.5 (2C, C-6<sub>D</sub>, 6<sub>E</sub>), 56.0 (C-2<sub>D</sub>), 55.3 (OCH<sub>3</sub>), 22.6 (C(O)CH<sub>3</sub>), 17.0 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). HRMS (MALDI) Calcd for C<sub>27</sub>H<sub>47</sub>NO<sub>19</sub> + Na: 858.3214. Found: 858.3206.

30                    **3,4-Di-O-benzyl-2-O-chloroacetyl-α/β-L-rhamnopyranose (128).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (Ir(I), 25 mg) was dissolved in dry THF (5 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of rhamnopyranoside(P. Westerduin, P. E. der Haan, M. J. Dees, J. H. van Boom, *Carbohydr. Res.* 1988, 180, 195-205) 127 (3.28 g, 7.12 mmol) in THF (30 mL) was degassed and added. The mixture was stirred overnight at rt, and a solution of iodine (3.6 g, 14.2 mmol) in a mixture of THF (70 mL) and water (20 mL) was added. The mixture was stirred at rt

for 1 h, then concentrated. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> and washed twice with 5% aq NaHSO<sub>4</sub>. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 9:1) to give **128** (2.53 g, 85%). <sup>1</sup>H NMR δ 7.40-7.28 (m, 10H, Ph), 5.57 (bd, 0.2H, H-2β), 5.45 (dd, 0.8H, J<sub>1,2</sub> = 2.0 Hz, H-2α), 5.13 (bd, 0.8H, H-1α), 4.92 (d, 1H, J = 10.9 Hz, OCH<sub>2</sub>α, OCH<sub>2</sub>β), 4.79 (d, 0.2H, J = 11.2 Hz, OCH<sub>2</sub>β), 4.74 (d, 1H, J = 11.2 Hz, OCH<sub>2</sub>α, H-1β), 4.65 (d, 0.8H, OCH<sub>2</sub>α), 4.64 (d, 0.2H, OCH<sub>2</sub>β), 4.58 (d, 0.8H, OCH<sub>2</sub>α), 4.54 (d, 0.2H, OCH<sub>2</sub>β), 4.30 (d, 0.2H, J = 15.1 Hz, CH<sub>2</sub>Clβ), 4.26 (d, 0.2H, CH<sub>2</sub>Clβ), 4.20 (s, 1.6H, CH<sub>2</sub>Clα), 4.08 (dd, 0.8H, J<sub>2,3</sub> = 3.3, J<sub>3,4</sub> = 9.6 Hz, H-3α), 4.04 (dq, 0.8H, J<sub>4,5</sub> = 9.5 Hz, H-5α), 3.66 (dd, 0.2H, J<sub>2,3</sub> = 3.2, J<sub>3,4</sub> = 8.7 Hz, H-3β), 3.44 (pt, 2H, H-4α, 5β, OH-1α, 1β), 3.38 (pt, 0.2H, J<sub>4,5</sub> = 9.5 Hz, H-4β), 1.37 (d, 0.6H, J<sub>5,6</sub> = 5.7 Hz, H-6β), 1.34 (d, 2.4H, J<sub>5,6</sub> = 6.2 Hz, H-6α); <sup>13</sup>C NMR δ 167.8 (COβ), 167.4 (COα), 138.6-128.2 (Ph), 93.0 (C-1β), 92.4 (C-1α), 80.3 (C-4α), 80.2 (C-3β), 79.6 (C-4β), 77.8 (C-3α), 75.9 (OCH<sub>2</sub>β), 75.8 (OCH<sub>2</sub>α), 72.5 (OCH<sub>2</sub>α), 72.3 (OCH<sub>2</sub>β), 71.9 (C-2-β), 71.7 (C-2-α), 68.2 (C-5α), 41.3 (CH<sub>2</sub>Clα, CH<sub>2</sub>Clβ), 18.3 (C-6α, 6β); FAB-MS for C<sub>22</sub>H<sub>25</sub>ClO<sub>6</sub> (M, 420.5) *m/z* 443.1 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>22</sub>H<sub>25</sub>ClO<sub>6</sub>: C, 62.78; H, 5.94%. Found: C, 62.92; H, 6.11%.

**3,4-Di-*O*-benzyl-2-*O*-chloroacetyl-α/β-L-rhamnopyranosyl**

**trichloroacetimidate (120).** (a) The hemiacetal **128** (700 mg, 1.66 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) and the solution was cooled to 0°C. Trichloroacetonitrile (1.7 mL) and DBU (26 μL) were added. The mixture was stirred at rt for 2 h. Toluene was added, and co-evaporated twice from the residue. The crude material was purified by flash chromatography (solvent B 4:1 + 0.1% Et<sub>3</sub>N) to give **120** as a white foam (687 mg, 73%, α/β: 4/1).

(b) The hemiacetal **128** (858 mg, 2.04 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (11 mL) and freshly activated K<sub>2</sub>CO<sub>3</sub> (1.1 g, 8.0 mmol) was added. The suspension was cooled to 0°C, and trichloroacetonitrile (1.0 mL) was added. The mixture was stirred vigorously at rt for 5 h. The suspension was filtered on a pad of Celite, and concentrated. The crude material was purified by flash chromatography (solvent B, 9:1 + 0.1% Et<sub>3</sub>N) to give **120** as a white foam (840 mg, 72%, α/β : 9/1 from the <sup>1</sup>H NMR spectrum). <sup>1</sup>H NMR (α-anomer) δ 8.71 (s, 1H, NH), 7.40-7.30 (m, 10H, Ph), 6.24 (d, 1H, J<sub>1,2</sub> = 1.8 Hz, H-1), 5.57 (dd, 1H, H-2), 4.94 (d, 1H, J = 10.8 Hz, OCH<sub>2</sub>), 4.76 (d, 1H, J = 11.2 Hz, OCH<sub>2</sub>), 4.67 (d, 1H, OCH<sub>2</sub>), 4.62 (d, 1H, OCH<sub>2</sub>), 4.22 (s, 2H, CH<sub>2</sub>Cl), 4.04 (dd, 1H, J<sub>2,3</sub> = 3.2 Hz, H-3), 3.99 (dq, 1H, J<sub>4,5</sub> = 9.6 Hz, H-5), 3.53 (pt, H, H-4), 1.37 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6); <sup>13</sup>C NMR (α-anomer) δ 166.9 (CO), 160.4 (C=NH), 138.4-128.3 (Ph), 95.2 (C-1), 91.1 (CCl<sub>3</sub>), 79.5 (C-4), 77.6 (C-3), 76.1, 72.9 (2C, OCH<sub>2</sub>), 71.2 (C-5), 69.8 (C-2), 41.1 (CH<sub>2</sub>Cl), 18.3 (C-6).

**Allyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranoside (122).** To a solution of the known

- 121(F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) (7.10 g, 8.55 mmol) in a mixture of CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and pyridine (5 mL) at 0°C was added chloroacetic anhydride (3.65 g, 21.3 mmol), and the mixture was stirred at this temperature for 2 h. TLC (solvent C, 9:1) showed the complete disappearance of the starting material. MeOH (10 mL) was added, and after 30 min, volatiles were evaporated. Column chromatography (solvent B, 1:0 → 4:1) of the crude yellow oil afforded **122** as a colourless foam (7.34 g, 95%). [ $\alpha$ ]<sub>D</sub> +47.5 (c 1.0); <sup>1</sup>H NMR  $\delta$  8.12-7.13 (m, 25H, Ph), 5.95 (m, 1H, CH=), 5.50-5.42 (m, 2H, J<sub>2,3</sub> = 3.6 Hz, H-2<sub>C</sub>, 3<sub>C</sub>), 5.37 (m, 1H, =CH<sub>2</sub>), 5.28 (m, 1H, =CH<sub>2</sub>), 4.96 (d, 1H, J = 11.0 Hz, OCH<sub>2</sub>), 4.93 (d, 1H, J<sub>1,2</sub> = 1.5 Hz, H-1<sub>C</sub>), 4.90 (d, 1H, J<sub>1,2</sub> = 3.3 Hz, H-1<sub>E</sub>), 4.87-4.81 (m, 3H, OCH<sub>2</sub>), 4.67 (d, 1H, J = 12.1 Hz, OCH<sub>2</sub>), 4.64 (d, 1H, J = 12.8 Hz, OCH<sub>2</sub>), 4.47 (d, 1H, J = 10.8 Hz, OCH<sub>2</sub>), 4.43 (d, 1H, J = 12.0 Hz, OCH<sub>2</sub>), 4.25 (m, 2H, OCH<sub>2</sub>), 4.09 (d, 1H, J = 15.5 Hz, CH<sub>2</sub>Cl), 3.99-3.93 (m, 3H, CH<sub>2</sub>Cl, H-5<sub>C</sub>, 3<sub>C</sub>), 3.84 (m, 1H, H-5<sub>E</sub>), 3.78-3.74 (m, 2H, H-6a<sub>E</sub>, 4<sub>E</sub>), 3.70 (pt, 1H, J<sub>4,5</sub> = J<sub>3,4</sub> = 9.3 Hz, H-4<sub>C</sub>), 3.58-3.54 (m, 2H, H-6b<sub>E</sub>, 2<sub>E</sub>), 1.50 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>C</sub>); <sup>13</sup>C NMR  $\delta$  167.0, 166.0 (2C, CO), 139.1-128.0 (Ph, All), 118.5 (All), 99.5 (C-1<sub>E</sub>), 96.8 (C-1<sub>C</sub>), 81.9 (C-3<sub>E</sub>), 81.0 (C-2<sub>E</sub>), 79.7 (C-4<sub>C</sub>), 77.7 (C-4<sub>E</sub>), 76.0, 75.4, 74.1, 73.8 (4C, OCH<sub>2</sub>), 73.5 (C-3<sub>C</sub>), 71.8 (C-5<sub>E</sub>), 70.9 (C-2<sub>C</sub>), 68.8 (OCH<sub>2</sub>), 68.1 (C-6<sub>E</sub>), 67.7 (C-5<sub>C</sub>), 41.5 (CH<sub>2</sub>Cl), 18.6 (C-6<sub>C</sub>); FAB-MS for C<sub>52</sub>H<sub>55</sub>O<sub>12</sub> (M, 906.5) *m/z* 929.3 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>52</sub>H<sub>55</sub>ClO<sub>12</sub>: C, 68.83; H, 6.11%. Found: C, 68.74; H, 6.19%.
- (2,3,4,6-Tetra-O-benzyl- $\alpha$ -D-glucopyranosyl)-(1→4)-2-O-benzoyl-3-O-chloroacetyl- $\alpha/\beta$ -L-rhamnopyranose (123).** A solution of **122** (7.21 g, 7.95 mmol) in THF (80 mL) containing activated iridium complex (60 mg) was treated as described for the preparation of **128**. The mixture was stirred at rt for 3 h, at which point a solution of iodine (4.0 g, 15.7 mmol) in a mixture of THF (90 mL) and water (24 mL) was added. The mixture was stirred at rt for 30 min, then concentrated. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> and washed twice with 5% aq NaHSO<sub>4</sub>, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 4:1) to give **123** (6.7 g, 97%) as a slightly yellow foam. <sup>1</sup>H NMR  $\delta$  8.10-7.09 (m, 25H, Ph), 5.47 (dd, 1H, J<sub>2,3</sub> = 3.5, J<sub>3,4</sub> = 9.3 Hz, H-3<sub>C</sub>), 5.41 (bs, 1H, H-2<sub>C</sub>), 5.03 (bs, 1H, H-1<sub>C</sub>), 4.94 (d, 1H, J = 10.9 Hz, OCH<sub>2</sub>), 4.87 (d, 1H, J<sub>1,2</sub> = 3.4 Hz, H-1<sub>E</sub>), 4.85 (d, 1H, OCH<sub>2</sub>), 4.80 (m, 2H, OCH<sub>2</sub>), 4.64 (m, 2H, OCH<sub>2</sub>), 4.45 (d, 1H, J = 10.7 Hz, OCH<sub>2</sub>), 4.41 (d, 1H, J = 12.1 Hz, OCH<sub>2</sub>), 4.16 (dq, 1H, J<sub>4,5</sub> = 9.3 Hz, H-5<sub>C</sub>), 4.09 (d, 1H, J = 15.6 Hz, CH<sub>2</sub>Cl), 3.96 (d, 1H, CH<sub>2</sub>Cl), 3.93 (pt, 1H, H-3<sub>E</sub>), 3.83 (m, 1H, H-5<sub>E</sub>), 3.77-3.68 (m, 2H, H-4<sub>E</sub>, 6a<sub>E</sub>), 3.65 (pt, 1H, H-4<sub>C</sub>), 3.54 (m, 2H, H-6b<sub>E</sub>, 2<sub>E</sub>), 1.48 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>C</sub>); <sup>13</sup>C NMR  $\delta$  167.0, 166.0 (2C, CO), 139.1-127.9 (Ph), 99.5 (C-1<sub>E</sub>), 92.3 (C-1<sub>C</sub>), 81.9 (C-3<sub>E</sub>), 81.0 (C-2<sub>E</sub>), 79.9 (C-4<sub>C</sub>), 77.6 (C-4<sub>E</sub>), 76.0, 75.6, 74.2, 74.1 (4C, OCH<sub>2</sub>), 72.1 (C-3<sub>C</sub>), 71.7 (C-4<sub>E</sub>), 71.1 (C-2<sub>C</sub>), 68.0 (C-6<sub>E</sub>), 67.5 (C-5<sub>C</sub>), 41.5 (CH<sub>2</sub>Cl), 18.9 (C-6<sub>C</sub>); FAB-MS for C<sub>49</sub>H<sub>51</sub>ClO<sub>12</sub> (M,

866.3)  $m/z$  889.3  $[M+Na]^+$ . Anal. Calcd for  $C_{49}H_{51}ClO_{12}$ : C, 67.85; H, 5.93%. Found: C, 67.72; H, 6.00%.

(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-2-*O*-benzoyl-3-*O*-chloroacetyl- $\alpha$ -L-rhamnopyranosyl trichloroacetimidate (**119**). Trichloroacetonitrile (1.1 mL, 10.9 mmol) and DBU (17  $\mu$ L) were added to a solution of the hemiacetal **123** (950 mg, 1.09 mmol) in dry  $CH_2Cl_2$  (8 mL), and the mixture was stirred at 0°C for 1.5 h. Toluene was added, and volatiles were evaporated. The residue was purified by flash chromatography (solvent B, 3:2 containing 0.1%  $Et_3N$ ) to give **119** (930 mg, 84%) as a colourless foam. Further elution gave some remaining starting material **123** (136 mg, 14%).  $[\alpha]_D +39.3$  (c 1.0);  $^1H$  NMR  $\delta$  8.76 (s, 1H, NH), 8.12-7.17 (m, 25H, Ph), 6.34 (d, 1H,  $J_{1,2} = 1.5$  Hz, H-1<sub>C</sub>), 5.67 (dd, 1H, H-2<sub>C</sub>), 5.54 (dd, 1H,  $J_{2,3} = 3.4$ ,  $J_{3,4} = 8.8$  Hz, H-3<sub>C</sub>), 4.98 (d, 1H, OCH<sub>2</sub>), 4.88 (d, 1H,  $J_{1,2} = 3.4$  Hz, H-1<sub>E</sub>), 4.84 (d, 1H,  $J = 11.1$  Hz, OCH<sub>2</sub>), 4.82 (d, 1H,  $J = 11.2$  Hz, OCH<sub>2</sub>), 4.65 (d, 1H, OCH<sub>2</sub>), 4.62 (d, 1H, OCH<sub>2</sub>), 4.44 (d, 1H,  $J = 11.4$  Hz, OCH<sub>2</sub>), 4.41 (d, 1H,  $J = 11.8$  Hz, OCH<sub>2</sub>), 4.14 (dq, 1H,  $J_{4,5} = 9.5$  Hz, H-5<sub>C</sub>), 4.11 (d, 1H,  $J = 15.5$  Hz, CH<sub>2</sub>Cl), 3.98 (d, 1H, CH<sub>2</sub>Cl), 3.94 (pt, 1H, H-3<sub>E</sub>), 3.83-3.71 (m, 4H, H-5<sub>E</sub>, 6a<sub>E</sub>, 4<sub>E</sub>, 4<sub>C</sub>), 3.56-3.51 (m, 2H, H-6b<sub>E</sub>, 2<sub>E</sub>), 1.51 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>C</sub>);  $^{13}C$  NMR  $\delta$  167.1, 165.7, 160.6 (3C, CO), 139.0-127.9 (Ph), 99.9 (C-1<sub>E</sub>), 95.2 (C-1<sub>C</sub>), 82.1 (C-3<sub>E</sub>), 80.9 (C-2<sub>E</sub>), 79.0 (C-4<sub>C</sub>), 77.6 (C-4<sub>E</sub>), 76.0, 75.6, 74.2, 73.8 (4C, OCH<sub>2</sub>), 73.0 (C-3<sub>C</sub>), 71.9 (C-5<sub>E</sub>), 70.7 (C-5<sub>C</sub>), 69.2 (C-2<sub>C</sub>), 68.0 (C-6<sub>E</sub>), 67.7 (C-5<sub>C</sub>), 41.4 (CH<sub>2</sub>Cl), 18.6 (C-6<sub>C</sub>). Anal. Calcd for  $C_{51}H_{51}Cl_4NO_{12}$ : C, 60.54; H, 5.08; N, 1.38%. Found: C, 60.49; H, 5.01; N, 1.34%.

Methyl (2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2-*O*-benzoyl-3-*O*-chloroacetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene- $\beta$ -D-glucopyranoside (**124**). The acceptor (L. A. Mulard, C. Costachel, P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, *19*, 849-877) **118** (500 mg, 1.82 mmol) was dissolved in  $CH_2Cl_2$  (5.5 mL) and 4Å-MS (300 mg) were added. The mixture was cooled to -60°C and stirred for 15 min. TMSOTf (35  $\mu$ L, mmol) and a solution of the disaccharide donor **119** (2.39 g, 2.36 mmol) in  $CH_2Cl_2$  (7.5 mL) were added. The mixture was stirred for 45 min while the cooling bath was coming back to rt, and for more 3 h at rt. The mixture was then heated at 65°C for 1 h 30 min.  $Et_3N$  was added and the mixture was stirred at rt for 20 min, then diluted with  $CH_2Cl_2$  and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 85:15  $\rightarrow$  1:1) to give **124** (1.64 g, 80%) as a white powder  $[\alpha]_D +55.1$  (c 1.0);  $^1H$  NMR  $\delta$  8.06-6.93 (m, 25H, Ph), 6.18 (d, 1H,  $J_{NH,2} = 7.3$  Hz, NH<sub>D</sub>), 5.40 (dd, 1H,  $J_{2,3} = 3.5$  Hz, H-3<sub>C</sub>), 5.38 (bs, 1H, H-2<sub>C</sub>), 4.98 (d, 1H,  $J_{1,2} = 8.3$  Hz, H-1<sub>D</sub>), 4.94 (bs, 1H, H-1<sub>C</sub>), 4.94 (d, 1H, OCH<sub>2</sub>), 4.93 (d, 1H,  $J_{1,2} = 3.4$  Hz, H-1<sub>E</sub>), 4.83 (d, 2H,  $J = 10.7$  Hz, OCH<sub>2</sub>), 4.81 (d, 1H,  $J = 10.6$  Hz, OCH<sub>2</sub>), 4.67 (d, 1H,  $J = 11.7$  Hz, OCH<sub>2</sub>), 4.62 (d, 1H,  $J = 11.4$  Hz, OCH<sub>2</sub>), 4.47 (m, 3H, H-3<sub>D</sub>, OCH<sub>2</sub>), 4.22 (dq, 1H,  $J_{4,5} = 9.4$ ,  $J_{5,6} = 6.2$  Hz, H-5<sub>C</sub>), 4.10 (d, 1H,  $J = 15.5$  Hz, CH<sub>2</sub>Cl),

3.96 (m, 2H, H-6a<sub>D</sub>, CH<sub>2</sub>Cl), 3.91 (pt, 1H, H-3<sub>E</sub>), 3.82 (m, 2H, H-5<sub>E</sub>, 6b<sub>D</sub>), 3.72 (m, 3H, H-6a<sub>E</sub>, 4<sub>E</sub>, 4<sub>C</sub>), 3.62 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 9.4 Hz, H-4<sub>D</sub>), 3.55 (m, 2H, H-6b<sub>E</sub>, 2<sub>E</sub>), 3.51 (s, 3H, OCH<sub>3</sub>), 3.41 (m, 1H, H-5<sub>D</sub>), 3.15 (m, 1H, H-2<sub>D</sub>), 2.04 (s, 3H, C(O)CH<sub>3</sub>), 1.51 (s, 3H, C(CH<sub>3</sub>)<sub>2</sub>), 1.42 (m, 6H, H-6<sub>C</sub>, C(CH<sub>3</sub>)<sub>2</sub>), 1.51 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>C</sub>); <sup>13</sup>C NMR δ 171.8, 167.3, 166.1 (3C, CO), 139.0-128.0 (Ph), 101.1 (C-1<sub>D</sub>, J<sub>CH</sub> < 164 Hz), 99.9 (C(CH<sub>3</sub>)<sub>2</sub>), 99.4 (C-1<sub>E</sub>, J<sub>CH</sub> > 165 Hz), 98.2 (C-1<sub>C</sub>, J<sub>CH</sub> = 172 Hz), 81.8 (C-3<sub>E</sub>), 80.9 (C-2<sub>E</sub>), 79.0 (C-4<sub>C</sub>\*), 77.7 (C-4<sub>E</sub>\*), 76.7 (C-3<sub>D</sub>), 75.9, 75.3, 74.2, 73.9 (4C, OCH<sub>2</sub>), 73.7 (C-4<sub>D</sub>), 73.4 (C-3<sub>C</sub>), 71.9 (C-5<sub>E</sub>), 71.2 (C-2<sub>C</sub>), 68.2 (C-6<sub>E</sub>), 67.8 (C-5<sub>C</sub>), 67.4 (C-5<sub>D</sub>), 62.7 (C-6<sub>D</sub>), 59.6 (C-2<sub>D</sub>), 57.6 (OCH<sub>3</sub>), 41.5 (CH<sub>2</sub>Cl), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 27.3 (C(O)CH<sub>3</sub>), 19.7 (C(CH<sub>3</sub>)<sub>2</sub>), 18.6 (C-6<sub>C</sub>); FAB-MS for C<sub>61</sub>H<sub>70</sub>ClNO<sub>17</sub> (M, 1123.4) *m/z* 1146.5 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>61</sub>H<sub>70</sub>ClNO<sub>17</sub>: C, 65.15; H, 6.27; N, 1.25%. Found: C, 65.13; H, 6.23; N, 1.22%.

**Methyl (2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene- $\beta$ -D-glucopyranoside (125).** To a solution of the fully protected **124** (1.40 g, 1.25 mmol) in a mixture of methanol (18 mL) and pyridine (18 mL) was added thiourea (951 mg, 12.5 mmol). The mixture was stirred at 65°C for 5 h at which time no TLC (solvent C, 4:1) that no starting material remained. Evaporation of the volatiles and co-evaporation of petroleum ether from the residue resulted in a crude solid which was taken up in a minimum of methanol. A large excess of CH<sub>2</sub>Cl<sub>2</sub> was added and the mixture was left to stand at 0°C for 1 h. The precipitate was filtrated on a pad of Celite and the filtrated was concentrated. Column chromatography of the residue (solvent C, 4:1) gave the trisaccharide acceptor **125** (1.28 g, 97%) as a white powder. [ $\alpha$ ]<sub>D</sub> +33.5 (c 1.0); <sup>1</sup>H NMR δ 8.10-6.96 (m, 25H, Ph), 6.09 (d, 1H, J<sub>NH,2</sub> = 7.9 Hz, NH<sub>D</sub>), 5.26 (dd, 1H, J<sub>1,2</sub> = 1.6, J<sub>2,3</sub> = 3.4 Hz, H-2<sub>C</sub>), 4.97 (m, 3H, H-1<sub>C</sub>, 1<sub>E</sub>, OCH<sub>2</sub>), 4.86 (m, 3H, H-1<sub>D</sub>, OCH<sub>2</sub>), 4.81 (d, 1H, OCH<sub>2</sub>), 4.72 (d, 1H, OCH<sub>2</sub>), 4.58 (d, 1H, J = 12.2 Hz, OCH<sub>2</sub>), 4.51 (d, 1H, J = 10.9 Hz, OCH<sub>2</sub>), 4.48 (d, 1H, J = 12.2 Hz, OCH<sub>2</sub>), 4.23 (pt, 1H, J<sub>2,3</sub> = J<sub>3,4</sub> = 9.4 Hz, H-3<sub>D</sub>), 4.18-4.10 (m, 2H, H-5<sub>C</sub>, 5<sub>E</sub>), 4.06-3.95 (m, 3H, H-3<sub>C</sub>, 3<sub>E</sub>, 6a<sub>D</sub>), 3.80 (pt, 1H, J<sub>5,6b</sub> = J<sub>6a,6b</sub> = 10.4 Hz, H-6b<sub>D</sub>), 3.66 (m, 2H, H-6a<sub>E</sub>, 6b<sub>E</sub>), 3.62 (dd, 1H, J<sub>2,3</sub> = 9.8, J<sub>1,2</sub> = 4.1 Hz, H-2<sub>E</sub>), 3.59 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 8.9 Hz, H-4<sub>E</sub>), 3.55 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 9.2 Hz, H-4<sub>D</sub>), 3.51 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 9.3 Hz, H-4<sub>C</sub>), 3.49 (s, 3H, OCH<sub>3</sub>), 2.22 (s, 3H, C(O)CH<sub>3</sub>), 1.90 (bs, 1H, OH), 1.49 (s, 3H, CMe<sub>2</sub>), 1.43 (s, 3H, CMe<sub>2</sub>), 1.40 (s, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>C</sub>); <sup>13</sup>C NMR δ 171.8, 166.6 (2C, CO), 138.9-128.1 (Ph), 101.6 (C-1<sub>D</sub>), 99.8 (C(CH<sub>3</sub>)<sub>2</sub>), 98.6 (C-1<sub>E</sub>\*), 98.3 (C-1<sub>C</sub>\*), 85.4 (C-4<sub>C</sub>), 82.0 (C-3<sub>E</sub>), 80.4 (C-2<sub>E</sub>), 78.2 (C-4<sub>E</sub>), 77.1 (C-3<sub>D</sub>), 75.9, 75.5, 74.2, 73.9 (4C, OCH<sub>2</sub>), 73.6 (C-4<sub>D</sub>\*), 73.5 (C-2<sub>C</sub>\*), 71.7 (C-5<sub>E</sub>), 69.0 (C-6<sub>E</sub>), 68.3 (C-3<sub>C</sub>), 67.5 (C-5<sub>D</sub>), 66.9 (C-5<sub>C</sub>), 62.7 (C-6<sub>D</sub>), 58.9 (C-2<sub>D</sub>), 57.5 (OCH<sub>3</sub>), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 24.0 (C(O)CH<sub>3</sub>), 19.7 (C(CH<sub>3</sub>)<sub>2</sub>), 18.2 (C-6<sub>C</sub>); FAB-MS for C<sub>59</sub>H<sub>69</sub>NO<sub>16</sub> (M, 1047.5) *m/z* 1070.4 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>70</sub>H<sub>76</sub>O<sub>16</sub>: C, 67.61; H, 6.64; N, 1.34%. Found: C, 67.46; H, 6.78; N, 1.24%.

**Methyl (3,4-Di-*O*-benzyl-2-*O*-chloroacetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl-3-*O*-chloroacetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene- $\beta$ -D-glucopyranoside (129).**

(a) The trisaccharide acceptor **125** (615 mg, 0.58 mmol) was dissolved in Et<sub>2</sub>O (10 mL) and the solution was cooled to  $-60^{\circ}\text{C}$ . TMSOTf (32  $\mu\text{L}$ ) and donor **120** (497 mg, 0.88 mmol) in Et<sub>2</sub>O (12 mL) were added, and the mixture was stirred for 1 h while the bath was slowly coming back to  $-20^{\circ}\text{C}$ . The mixture was stirred for 4 h at this temperature, then at  $0^{\circ}\text{C}$  overnight. More **120** (50 mg, 88  $\mu\text{mol}$ ) was added, and the mixture was stirred at rt for 3 h more at  $0^{\circ}\text{C}$ . Et<sub>3</sub>N was added, and the mixture was concentrated. Column chromatography of the residue (solvent B, 9:1  $\rightarrow$  1:1) gave the orthoester **135** (44 mg, 5%) then the fully protected **129** (445 mg, 52%) contaminated with the trimethylsilyl side product **126** (**129/126**: 9/1) together with a mixture of **129** and **135** (65 mg, 8%), and the starting **125** (27 mg, 4%). An analytical sample of compound **129** had  $[\alpha]_{\text{D}} +17.9$  (c 1.0); <sup>1</sup>H NMR  $\delta$  8.07-7.12 (m, 35H, Ph), 5.96 (d, 1H,  $J_{\text{NH},2} = 7.9$  Hz, NH), 5.82 (m, 1H, H-2<sub>B</sub>), 5.33 (dd, 1H,  $J_{1,2} = 1.8$ ,  $J_{2,3} = 3.2$  Hz, H-2<sub>C</sub>), 5.07 (d, 1H,  $J_{1,2} = 3.2$  Hz, H-1<sub>E</sub>), 5.05 (d, 1H,  $J_{1,2} = 1.7$  Hz, H-1<sub>B</sub>), 4.98 (d, 1H, OCH<sub>2</sub>), 4.97 (bs, 1H, H-1<sub>C</sub>), 4.91-4.78 (m, 5H, H-1<sub>D</sub>, OCH<sub>2</sub>), 4.64 (d, 1H,  $J = 11.6$  Hz, OCH<sub>2</sub>), 4.60-4.45 (m, 5H, OCH<sub>2</sub>), 4.36 (d, 1H,  $J = 11.9$  Hz, OCH<sub>2</sub>), 4.26 (pt, 1H,  $J_{2,3} = J_{3,4} = 9.5$  Hz, H-3<sub>D</sub>), 4.17 (dd, 1H,  $J_{2,3} = 3.4$  Hz, H-3<sub>C</sub>), 4.16 (d, 1H,  $J = 15.1$  Hz, CH<sub>2</sub>Cl), 4.11 (d, 1H, CH<sub>2</sub>Cl), 4.10 (dq, 1H,  $J_{4,5} = 9.1$ ,  $J_{5,6} = 6.3$  Hz, H-5<sub>C</sub>), 4.06 (m, 1H, H-5<sub>E</sub>), 4.00 (pt, 1H,  $J_{3,4} = J_{2,3} = 9.4$  Hz, H-3<sub>E</sub>), 3.97 (dd, 1H,  $J_{5,6a} = 5.3$ ,  $J_{6a,6b} = 10.8$  Hz, 6a<sub>D</sub>), 3.89 (m, 1H, H-6a<sub>E</sub>), 3.88-3.68 (m, 4H, H-6b<sub>E</sub>, 6b<sub>D</sub>, 4<sub>C</sub>, 3<sub>B</sub>), 3.67 (m, 1H, H-5<sub>B</sub>), 3.58 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.4$  Hz, H-4<sub>D</sub>), 3.52 (dd, 1H,  $J_{1,2} = 3.3$ ,  $J_{2,3} = 9.8$  Hz, H-2<sub>E</sub>), 3.49 (s, 3H, OCH<sub>3</sub>), 3.39 (m, 1H, H-5<sub>D</sub>), 3.30 (m, 2H, H-2<sub>D</sub>, 4<sub>B</sub>), 2.12 (s, 3H, C(O)CH<sub>3</sub>), 1.52 (s, 3H, C(CH<sub>3</sub>)<sub>2</sub>), 1.42 (s, 3H, C(CH<sub>3</sub>)<sub>2</sub>), 1.33, 0.96 (2d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>B</sub>, 6<sub>C</sub>); <sup>13</sup>C NMR  $\delta$  171.9, 167.0, 166.3 (3C, CO), 138.8-128.0 (Ph), 101.4 (C-1<sub>D</sub>,  $J_{\text{CH}} = 164$  Hz), 99.9 (C(CH<sub>3</sub>)<sub>2</sub>), 99.3 (C-1<sub>C</sub>,  $J_{\text{CH}} = 168$  Hz), 98.3 (C-1<sub>E</sub>,  $J_{\text{CH}} = 168$  Hz), 97.9 (C-1<sub>B</sub>,  $J_{\text{CH}} = 171$  Hz), 82.1 (C-3<sub>E</sub>), 81.8 (C-2<sub>E</sub>), 80.4 (bs, C-3<sub>B</sub>), 80.0 (C-4<sub>C</sub>), 78.8 (bs, C-4<sub>E</sub>\*), 78.3 (C-4<sub>B</sub>\*), 77.7 (C-3<sub>C</sub>\*), 76.9 (C-3<sub>D</sub>), 75.9, 75.5, 75.3, 74.3 (4C, OCH<sub>2</sub>), 73.4 (C-4<sub>D</sub>), 73.2 (OCH<sub>2</sub>), 72.7 (C-2<sub>B</sub>), 72.1 (C-5<sub>E</sub>), 69.1 (C-5<sub>C</sub>), 67.7 (C-5<sub>D</sub>\*), 67.6 (C-5<sub>B</sub>\*), 62.7 (C-6<sub>D</sub>), 59.1 (C-2<sub>D</sub>), 57.5 (OCH<sub>3</sub>), 41.4 (CH<sub>2</sub>Cl), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 24.0 (C(O)CH<sub>3</sub>), 19.7 (C(CH<sub>3</sub>)<sub>2</sub>), 18.8, 18.2 (2C, C-6<sub>B</sub>, 6<sub>C</sub>); FAB-MS for C<sub>81</sub>H<sub>92</sub>NClO<sub>21</sub> (M, 1449.5)  $m/z$  1472.7  $[\text{M}+\text{Na}]^+$ . Anal. Calcd for C<sub>81</sub>H<sub>92</sub>NClO<sub>21</sub>: C, 67.05; H, 6.39; N, 0.97%. Found: C, 66.21; H, 6.46; 1.01%.

Compound **135** had  $[\alpha]_{\text{D}} +26.7$  (c 0.8); <sup>1</sup>H NMR  $\delta$  8.07-7.15 (m, 35H, Ph), 5.47 (d, 1H,  $J_{\text{NH},2} = 7.4$  Hz, NH<sub>D</sub>), 5.45 (bs, 1H, H-2<sub>C</sub>), 5.42 (d, 1H,  $J_{1,2} = 2.3$  Hz, H-1<sub>B</sub>), 5.24 (d, 1H,  $J_{1,2} = 3.4$  Hz, H-1<sub>E</sub>), 4.94 (d, 1H,  $J_{1,2} = 8.2$  Hz, H-1<sub>D</sub>), 4.91-4.82 (m, 7H, H-1<sub>C</sub>, OCH<sub>2</sub>), 4.80 (d, 1H,  $J = 11$  Hz, OCH<sub>2</sub>), 4.75 (d, 1H,  $J = 11.6$  Hz, OCH<sub>2</sub>), 4.68 (dd, 1H,  $J_{1,2} = 2.4$ ,  $J_{2,3} = 4.0$  Hz, H-2<sub>B</sub>), 4.65-4.47 (m, 4H, OCH<sub>2</sub>), 4.44-4.32 (m, 4H, H-5<sub>E</sub>, 3<sub>D</sub>, 3<sub>C</sub>, OCH<sub>2</sub>),

4.15 (m, 1H, H-5<sub>C</sub>), 4.05 (pt, 1H,  $J_{2,3} = J_{3,4} = 9.5$  Hz, H-3<sub>E</sub>), 4.03 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.4$  Hz, H-4<sub>C</sub>), 3.94 (dd, 1H,  $J_{5,6a} = 5.3$ ,  $J_{6a,6b} = 10.7$  Hz, H-6a<sub>D</sub>), 3.83-3.75 (m, 4H, H-6a<sub>E</sub>, 6b<sub>D</sub>, CH<sub>2</sub>Cl), 3.74-3.70 (m, 3H, H-4<sub>E</sub>, 6<sub>E</sub>, 3<sub>B</sub>), 3.65 (dd, 1H,  $J_{1,2} = 3.4$ ,  $J_{2,3} = 9.4$  Hz, H-2<sub>E</sub>), 3.48 (pt, 2H, H-4<sub>B</sub>, 4<sub>D</sub>), 3.46 (s, 3H, OCH<sub>3</sub>), 3.38 (m, 1H, H-5<sub>D</sub>), 3.22 (dq, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.2$  Hz, H-5<sub>B</sub>), 2.88 (m, 1H, H-2<sub>D</sub>), 1.90 (s, 3H, C(O)CH<sub>3</sub>), 1.42 (s, 3H, C(CH<sub>3</sub>)<sub>2</sub>), 1.36 (s, 6H, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>C</sub>), 1.30 (s, 3H,  $J_{5,6} = 6.3$  Hz, H-6<sub>B</sub>); <sup>13</sup>C NMR δ 171.8, 166.4 (2C, CO), 139.1-122.5 (Ph), 101.0 (C-1<sub>D</sub>,  $J_{CH} = 165$  Hz), 99.7 (C(CH<sub>3</sub>)<sub>2</sub>), 98.3 (C-1<sub>C</sub>,  $J_{CH} = 172$  Hz), 97.8 (bs, C-1<sub>E</sub>,  $J_{CH} = 170$  Hz), 97.5 (C-1<sub>B</sub>,  $J_{CH} = 176$  Hz), 82.2 (C-3<sub>E</sub>), 80.7 (C-2<sub>E</sub>), 79.3 (bs, C-4<sub>B</sub>), 78.8 (C-3<sub>B</sub>), 78.1 (bs, C-4<sub>E</sub>), 77.3 (C-2<sub>B</sub>), 76.2 (bs, C-3<sub>C</sub>), 75.8, 75.6, 74.9, 74.6, 73.9 (6C, C-4<sub>C</sub>, OCH<sub>2</sub>), 73.5 (2C, C-4<sub>D</sub>, 2<sub>C</sub>), 71.4 (OCH<sub>2</sub>), 71.0 (C-3<sub>D</sub>), 70.7 (2C, C-5<sub>E</sub>, 5<sub>B</sub>), 69.0 (C-5<sub>C</sub>), 68.8 (C-6<sub>E</sub>), 67.2 (C-5<sub>D</sub>), 62.5 (C-6<sub>D</sub>), 60.0 (C-2<sub>D</sub>), 57.6 (OCH<sub>3</sub>), 46.9 (CH<sub>2</sub>Cl), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 23.9 (C(O)CH<sub>3</sub>), 19.7 (C(CH<sub>3</sub>)<sub>2</sub>), 19.0 (C-6<sub>B</sub>), 18.4 (C-6<sub>C</sub>); FAB-MS for C<sub>81</sub>H<sub>92</sub>NCIO<sub>21</sub> (M, 1449.5)  $m/z$  1472.7 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>81</sub>H<sub>92</sub>NCIO<sub>21</sub>·H<sub>2</sub>O: C, 66.23; H, 6.34; N, 0.96%. Found: C, 66.11; H, 6.62; N, 0.85%.

**Methyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene-β-D-glucopyranoside (130).** The trisaccharide acceptor **125** (500 mg, 0.47 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and the solution was cooled to -40°C. TMSOTf (21 μL) and donor **105** (328 mg, 0.62 mmol) were added and the mixture was left under stirring while the bath was slowly coming back to rt. After 5 h, more **105** (50 mg, 94 μmol) was added and the mixture was stirred at rt for 1 h more at rt. Et<sub>3</sub>N was added and the mixture was concentrated. Column chromatography of the residue (solvent B, 4:1 → 1:1) gave the fully protected **130** (484 mg, 72%) slightly contaminated with the corresponding trimethylsilyl side-product **126**. The **130**:**126** ratio was estimated to be 85:15 from the <sup>1</sup>H NMR spectrum. Eluting next was some residual starting **125** (45 mg, 9%), thus based on the consumed acceptor, the estimated yield of contaminated **130** was 79%. An analytical sample of **130** had [α]<sub>D</sub> +15.9 (c 0.8); <sup>1</sup>H NMR: δ 8.09-7.14 (m, 35H, Ph), 6.04 (bs, 1H, NH<sub>D</sub>), 5.76 (m, 1H, H-2<sub>B</sub>), 5.37 (dd, 1H,  $J_{1,2} = 1.9$ ,  $J_{2,3} = 2.8$  Hz, H-2<sub>C</sub>), 5.11 (d, 1H,  $J_{1,2} = 3.1$  Hz, H-1<sub>E</sub>), 5.06 (d, 1H, H-1<sub>B</sub>), 4.96 (bs, 1H, H-1<sub>C</sub>), 5.02-4.82 (m, 7H, H-1<sub>D</sub>, OCH<sub>2</sub>), 4.69-4.37 (m, 6H, OCH<sub>2</sub>), 4.28 (pt, 1H,  $J_{2,3} = J_{3,4} = 9.5$  Hz, H-3<sub>D</sub>), 4.15 (dd, 1H,  $J_{2,3} = 3.3$ ,  $J_{3,4} = 9.4$  Hz, H-3<sub>C</sub>), 4.13-3.93 (m, 5H, H-5<sub>E</sub>, 6a<sub>E</sub>, 3<sub>E</sub>, 5<sub>C</sub>, 6a<sub>D</sub>), 3.87-3.76 (m, 5H, H-4<sub>E</sub>, 6b<sub>E</sub>, 3<sub>B</sub>, 4<sub>C</sub>, 6b<sub>D</sub>), 3.68 (dq, 1H,  $J_{4,5} = 9.5$  Hz, H-5<sub>B</sub>), 3.57 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.4$  Hz, H-4<sub>D</sub>), 3.54 (dd, 1H,  $J_{2,3} = 3.2$  Hz, H-2<sub>E</sub>), 3.48 (s, 3H, OCH<sub>3</sub>), 3.40 (m, 1H, H-5<sub>D</sub>), 3.34 (pt, 1H,  $J_{3,4} = 9.7$  Hz, H-4<sub>B</sub>), 3.27 (m, 1H, H-2<sub>D</sub>), 2.18, 2.13 (2s, 6H, C(O)CH<sub>3</sub>), 1.51, 1.42 (2s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 1.33 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>C</sub>), 0.98 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>B</sub>); <sup>13</sup>C NMR δ 171.9, 170.5, 166.3 (3C, CO), 139.3-127.7 (Ph), 101.3 (C-1<sub>D</sub>), 99.9 (C(CH<sub>3</sub>)<sub>2</sub>), 99.6 (C-1<sub>B</sub>), 98.4 (C-1<sub>E</sub>), 98.0 (C-1<sub>C</sub>), 82.1 (C-3<sub>E</sub>), 81.8 (C-2<sub>E</sub>), 80.3 (2C, C-3<sub>C</sub>, 4<sub>B</sub>), 78.7 (bs, C-4<sub>C</sub>), 78.2 (C-3<sub>B</sub>\*), 77.7 (C-

4<sub>E</sub>\*), 76.9 (bs, C-3<sub>D</sub>), 75.9, 75.4, 75.3, 74.3 (4C, OCH<sub>2</sub>), 73.4 (C-4<sub>D</sub>), 73.3 (OCH<sub>2</sub>), 72.7 (C-2<sub>C</sub>), 72.1 (C-5<sub>E</sub>), 70.9 (OCH<sub>2</sub>), 69.0 (3C, C-2<sub>B</sub>, 5<sub>B</sub>, 6<sub>E</sub>), 67.8 (C-5<sub>C</sub>), 67.6 (C-5<sub>D</sub>), 62.7 (C-6<sub>D</sub>), 59.2 (C-2<sub>D</sub>), 57.5 (OCH<sub>3</sub>), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 24.0, 21.6 (2C, C(O)CH<sub>3</sub>), 19.7 (C(CH<sub>3</sub>)<sub>2</sub>), 18.9 (C-6<sub>C</sub>), 18.2 (C-6<sub>B</sub>). FAB-MS for C<sub>81</sub>H<sub>93</sub>NO<sub>21</sub> (M, 1415) *m/z* 1438.6 [M+Na]<sup>+</sup>.

**Methyl (3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene- $\beta$ -D-glucopyranoside (131).** (a) Thiourea (22 mg, 0.29 mmol) was added to the chloroacetylated **129** (83 mg, 57  $\mu$ mol) in MeOH/pyridine (1/1, 2.8 mL), and the mixture was heated overnight at 65°C. Volatiles were evaporated, and the solid residue thus obtained was taken up in the minimum of MeOH. CH<sub>2</sub>Cl<sub>2</sub> was added, and the suspension was left standing at 0°C for 1 h. The precipitate was filtered on a pad of Celite, and the filtrate was concentrated. Column chromatography of the residue (solvent B, 9:1  $\rightarrow$  1:1) gave the tetrasaccharide acceptor **131** (74 mg, 94%).

(b) The monoacetylated **130** (52 mg, 37  $\mu$ mol) was dissolved in a mixture of EtOH (10 mL) and CH<sub>2</sub>Cl<sub>2</sub> (100  $\mu$ L). A freshly prepared 0.4 M ethanolic solution of guanidine (92  $\mu$ L, 37  $\mu$ mol) was added and the mixture was stirred at rt overnight. Volatiles were evaporated, and the residue taken up in CH<sub>2</sub>Cl<sub>2</sub> was washed with water. The organic phase was dried and concentrated. Column chromatography of the crude product gave **131** (42 mg, 83%) as a glassy solid. Compound **131** had [ $\alpha$ ]<sub>D</sub> +27.3 (c 1.0); <sup>1</sup>H NMR  $\delta$  8.24-6.88 (m, 35H, Ph), 5.90 (bs, 1H, NH<sub>D</sub>), 5.29 (bs, 1H, H-2<sub>C</sub>), 5.14 (d, 1H, J<sub>1,2</sub> = 3.0 Hz, H-1<sub>E</sub>), 5.06 (d, 1H, J<sub>1,2</sub> = 1.6 Hz, H-1<sub>B</sub>), 5.00-4.95 (m, 3H, H-1<sub>D</sub>, 1<sub>C</sub>, OCH<sub>2</sub>), 4.88-4.46 (m, 9H, OCH<sub>2</sub>), 4.31 (pt, 1H, J<sub>2,3</sub> = J<sub>3,4</sub> = 9.4 Hz, H-3<sub>D</sub>), 4.24 (bs, 1H, H-2<sub>B</sub>), 4.14-3.08 (m, 3H, H-3<sub>C</sub>, 5<sub>C</sub>, 5<sub>E</sub>), 4.02 (pt, 1H, J<sub>2,3</sub> = J<sub>3,4</sub> = 9.3 Hz, H-3<sub>E</sub>), 3.97 (dd, 1H, J<sub>5,6a</sub> = 5.2, J<sub>6a,6b</sub> = 10.7 Hz, 6a<sub>D</sub>), 3.80 (m, 2H, H-4<sub>C</sub>, 6b<sub>D</sub>), 3.71 (m, 2H, H-6a<sub>E</sub>, 6b<sub>E</sub>), 3.66 (pt, 1H, J<sub>4,5</sub> = 9.5 Hz, H-4<sub>E</sub>), 3.61-3.55 (m, 4H, H-3<sub>B</sub>, 2<sub>E</sub>, 5<sub>B</sub>, 4<sub>D</sub>), 3.50 (s, 3H, OCH<sub>3</sub>), 3.42-3.36 (m, 2H, H-5<sub>D</sub>, 4<sub>B</sub>), 3.20 (m, 1H, H-2<sub>D</sub>), 2.85 (bs, 1H, OH), 2.10 (s, 3H, C(O)CH<sub>3</sub>), 1.51, 1.41 (2s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 1.33 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>C</sub>), 1.15 (s, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>B</sub>); <sup>13</sup>C NMR  $\delta$  171.7, 166.3 (2C, CO), 139.0-127.8 (Ph), 103.1 (C-1<sub>B</sub>), 101.2 (C-1<sub>D</sub>), 99.8 (C(CH<sub>3</sub>)<sub>2</sub>), 98.2, 98.1 (2C, C-1<sub>E</sub>, 1<sub>C</sub>), 82.0 (C-3<sub>E</sub>), 81.5 (C-3<sub>B</sub>\*), 80.6 (C-4<sub>B</sub>), 79.4 (C-2<sub>E</sub>\*), 79.1 (2C, C-4<sub>C</sub>, 3<sub>C</sub>), 78.2 (C-4<sub>B</sub>), 76.8 (C-3<sub>D</sub>), 76.0, 75.5, 74.5, 74.2 (4C, OCH<sub>2</sub>), 73.9 (C-2<sub>C</sub>), 73.7 (OCH<sub>2</sub>), 73.5 (C-4<sub>D</sub>), 72.1 (OCH<sub>2</sub>), 71.6 (C-5<sub>E</sub>), 69.0 (C-6<sub>E</sub>), 68.7 (2C, C-2<sub>B</sub>, 5<sub>B</sub>), 67.9 (C-5<sub>C</sub>), 67.5 (C-5<sub>D</sub>), 62.7 (C-6<sub>D</sub>), 59.4 (C-2<sub>D</sub>), 57.5 (OCH<sub>3</sub>), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 24.0 (C(O)CH<sub>3</sub>), 19.7 (C(CH<sub>3</sub>)<sub>2</sub>), 19.0 (C-6<sub>C</sub>), 18.3 (C-6<sub>B</sub>); FAB-MS for C<sub>79</sub>H<sub>91</sub>NO<sub>20</sub> (M, 1373) *m/z* 1396.5 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>79</sub>H<sub>91</sub>NO<sub>20</sub>·0.5 H<sub>2</sub>O: C, 68.56; H, 6.65; N, 1.01%. Found: C, 68.53; H, 6.71; N, 1.01%.



**Methyl (2-*O*-Acetyl-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl-3-*O*-chloroacetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-3,4-*O*-isopropylidene- $\beta$ -D-glucopyranoside (132).** Activated 4Å molecular sieves and TMSOTf (16  $\mu$ L) were added to a solution of the tetrasaccharide acceptor 131 (406 mg, 0.29 mmol) in Et<sub>2</sub>O (10 mL), and the mixture was stirred at -60°C for 30 min. The donor 105 (234 mg, 0.44 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (7 mL) was added, and the mixture was stirred for 1 h while the bath temperature was reaching rt. After a further 1 h at this temperature, more 105 (50 mg, 94  $\mu$ mol) was added, and the mixture was stirred for 1 h before Et<sub>3</sub>N was added. Filtration through a pad of Celite and evaporation of the volatiles gave a residue which was column chromatographed twice (solvent B, 4:1; then solvent A, 17:3) to give 132 (262 mg, 52%) as a white powder;  $[\alpha]_D^{+25.9}$  (c 1.0); <sup>1</sup>H NMR  $\delta$  8.07-7.13 (m, 45H, Ph), 6.03 (bs, 1H, NH<sub>D</sub>), 5.59 (bs, 1H, H-2<sub>A</sub>), 5.35 (bs, 1H, H-2<sub>C</sub>), 5.16 (bs, 1H, H-1<sub>E</sub>), 5.13 (bs, 1H, H-1<sub>A</sub>), 5.06 (bs, 1H, H-1<sub>B</sub>), 5.02-4.97 (m, 4H, H-1<sub>D</sub>, 1<sub>C</sub>, OCH<sub>2</sub>), 4.91-4.50 (m, 12H, OCH<sub>2</sub>), 4.44-4.32 (m, 4H, H-2<sub>B</sub>, 3<sub>D</sub>, OCH<sub>2</sub>), 4.20-3.96 (m, 7H, H-5<sub>E</sub>, 5<sub>A</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 6a<sub>D</sub>, 5<sub>C</sub>, 3<sub>A</sub>), 3.87-3.68 (m, 6H, H-4<sub>E</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 6b<sub>D</sub>, 4<sub>C</sub>, 3<sub>B</sub>), 3.64-3.47 (m, 7H, H-5<sub>B</sub>, 4<sub>D</sub>, 2<sub>E</sub>, 4<sub>A</sub>, OCH<sub>3</sub>), 3.42 (m, 1H, H-5<sub>D</sub>), 3.34 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 9.3 Hz, H-4<sub>B</sub>), 3.17 (m, 1H, H-2<sub>D</sub>), 2.13 (s, 3H, C(O)CH<sub>3</sub>), 1.49 (s, 3H, C(CH<sub>3</sub>)<sub>2</sub>), 1.43 (s, 6H, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>C</sub>), 1.33 (d, 3H, J<sub>5,6</sub> = 6.1 Hz, H-6<sub>A</sub>), 1.01 (d, 3H, J<sub>5,6</sub> = 5.8 Hz, H-6<sub>B</sub>); <sup>13</sup>C NMR  $\delta$  171.9, 170.3, 166.3 (3C, CO), 139.2-127.6 (Ph), 101.5 (bs, C-1<sub>B</sub>, J<sub>CH</sub> = 171 Hz), 101.2 (C-1<sub>D</sub>, J<sub>CH</sub> = 163 Hz), 99.8 (C(CH<sub>3</sub>)<sub>2</sub>), 99.7 (C-1<sub>A</sub>, J<sub>CH</sub> = 171 Hz), 97.9 (2C, C-1<sub>E</sub>, 1<sub>C</sub>, J<sub>CH</sub> = 172, J<sub>CH</sub> = 169 Hz), 82.4 (C-3<sub>E</sub>), 82.1 (C-2<sub>E</sub>), 80.5 (C-4<sub>A</sub>), 80.2 (bs, C-3<sub>C</sub>), 80.1 (C-4<sub>B</sub>), 79.4, 78.1, 78.0 (4C, C-3<sub>B</sub>, 4<sub>E</sub>, 3<sub>A</sub>, 4<sub>C</sub>), 76.6 (bs, C-3<sub>D</sub>), 75.9, 75.8, 75.4 (3C, OCH<sub>2</sub>), 74.8 (2C, C-2<sub>B</sub>, OCH<sub>2</sub>), 73.5 (C-4<sub>D</sub>), 73.4 (OCH<sub>2</sub>), 73.2 (C-2<sub>C</sub>), 72.1 (OCH<sub>2</sub>), 71.8 (C-5<sub>A</sub>), 71.2 (OCH<sub>2</sub>), 69.4 (C-2<sub>A</sub>), 69.2 (C-5<sub>B</sub>), 68.9 (C-6<sub>E</sub>), 68.7 (C-5<sub>C</sub>), 67.8 (C-5<sub>E</sub>), 67.5 (C-5<sub>D</sub>), 62.7 (C-6<sub>D</sub>), 59.6 (bs, C-2<sub>D</sub>), 57.6 (OCH<sub>3</sub>), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 24.0, 21.4 (2C, C(O)CH<sub>3</sub>), 19.7 (C(CH<sub>3</sub>)<sub>2</sub>), 19.1 (C-6<sub>A</sub>), 18.8 (C-6<sub>C</sub>), 18.2 (C-6<sub>B</sub>); FAB-MS for C<sub>101</sub>H<sub>115</sub>NO<sub>25</sub> (M, 1741.7) *m/z* 1765.9 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>101</sub>H<sub>115</sub>NO<sub>25</sub>: C, 69.60; H, 6.65; N, 0.80%. Found: C, 69.56; H, 6.75; N, 0.73%.

**Methyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (102).** 50% aq TFA (1 mL) was added at 0°C to a solution of the fully protected pentasaccharide 132 (155 mg, 89  $\mu$ mol) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4 mL). After 1 h at this temperature, volatiles were evaporated. The residue (crude 133) was taken up in 0.5M methanolic sodium methoxide (8 mL) and the mixture was heated overnight at 55°C. Neutralisation with Dowex X8 (H<sup>+</sup>), evaporation of the volatiles, and column chromatography of the residue gave 134 (121 mg, 98%). Compound 134 (111 mg, 81  $\mu$ mol) was dissolved in a mixture of ethanol (13 mL) and ethyl acetate (2.6 mL) containing

1N aq HCl (130  $\mu$ L). Palladium on charcoal (130 mg) was added and the suspension was stirred under a hydrogen atmosphere for 2 h. Filtration of the catalyst and reverse phase chromatography gave the target pentasaccharide (60 mg, 88%) as a slightly yellow foam. RP-HPLC purification followed by freeze-drying gave pure **102** (36 mg). Compound **102** had Rt: 9.63 min (solvent F, 100:0  $\rightarrow$  80:20 over 20 min);  $[\alpha]_D -18.6$  (c 1.0, methanol);  $^1\text{H}$  NMR  $\delta$  5.13 (d, 1H,  $J_{1,2} = 3.7$  Hz, H-1<sub>E</sub>), 4.98 (bs, 1H, H-1<sub>B</sub>), 4.90 (d, 1H,  $J_{1,2} = 1.4$  Hz, H-1<sub>A</sub>), 4.72 (d, 1H,  $J_{1,2} = 1.4$  Hz, H-1<sub>C</sub>), 4.39 (d, 1H,  $J_{1,2} = 8.6$  Hz, H-1<sub>D</sub>), 4.09 (dq, 1H,  $J_{4,5} = 9.2$  Hz, H-5<sub>C</sub>), 4.00 (m, 2H, H-2<sub>B</sub>, 2<sub>A</sub>), 3.94-3.79 (m, 7H, H-5<sub>E</sub>, 2<sub>C</sub>, 3<sub>C</sub>, 6a<sub>E</sub>, 6a<sub>D</sub>, 2<sub>D</sub>, 3<sub>A</sub>), 3.76-3.65 (m, 7H, H-4<sub>C</sub>, 3<sub>E</sub>, 6b<sub>E</sub>, 6b<sub>D</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 3<sub>B</sub>), 3.52 (pt, 1H,  $J_{3,4} = 8.8$  Hz, H-3<sub>D</sub>), 3.49-3.33 (m, 9H, H-4<sub>D</sub>, 2<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 5<sub>D</sub>, 4<sub>E</sub>, OCH<sub>3</sub>), 1.98 (s, 3H, C(O)CH<sub>3</sub>), 1.27 (d, 3H,  $J_{5,6} = 6.3$  Hz, H-6<sub>C</sub>), 1.24, 1.23 (d, 3H, H-6<sub>A</sub>, 6<sub>B</sub>);  $^{13}\text{C}$  NMR  $\delta$  172.3 (CO), 100.7 (C-1<sub>A</sub>,  $J_{\text{CH}} = 171$  Hz), 99.6 (2C, C-1<sub>D</sub>, 1<sub>B</sub>,  $J_{\text{CH}} = 163$ ,  $J_{\text{CH}} = 170$  Hz), 99.2 (C-1<sub>C</sub>,  $J_{\text{CH}} = 170$  Hz), 95.7 (bs, C-1<sub>E</sub>,  $J_{\text{CH}} = 170$  Hz), 82.0 (C-3<sub>D</sub>), 79.1 (C-2<sub>B</sub>), 79.4 (bs, C-3<sub>C</sub>), 76.4 (C-5<sub>D</sub>\*), 75.4 (bs, C-4<sub>C</sub>), 73.0 (C-3<sub>E</sub>), 72.4 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 72.2 (C-5<sub>E</sub>), 71.7 (C-2<sub>E</sub>), 71.1 (C-2<sub>C</sub>), 70.4, 70.1, 70.0 (4C, C-2<sub>A</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 4<sub>E</sub>), 69.7, 69.6, 69.3 (3C, C-5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>), 68.8 (C-4<sub>D</sub>), 61.2, 61.0 (2C, C-6<sub>D</sub>, 6<sub>E</sub>), 57.4 (OCH<sub>3</sub>), 55.4 (C-2<sub>D</sub>), 22.6 (C(O)CH<sub>3</sub>), 18.2 (C-6<sub>C</sub>), 17.2, 17.0 (C-6<sub>A</sub>, 6<sub>B</sub>); HRMS (MALDI) Calcd for C<sub>33</sub>H<sub>57</sub>NO<sub>23</sub> + Na: 858.3219. Found: 858.3089.

**Methyl (2-O-Acetyl-3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[(2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)]-(2-O-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (136).** 50% aq TFA (400  $\mu$ L) was added to a solution of the fully protected tetrasaccharide **130** (57 mg, 40  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) at 0°C, and the mixture was stirred overnight at this temperature. Volatiles were evaporated and the residue was purified by column chromatography (solvent B, 1:1) to give diol **136** (47 mg, 85%).  $[\alpha]_D +19.5$  (c 0.9);  $^1\text{H}$  NMR  $\delta$  8.10-7.16 (m, 35H, Ph), 5.80 (d, 1H,  $J = 8.8$  Hz, NH<sub>D</sub>), 5.66 (m, 1H, H-2<sub>B</sub>), 5.39 (pt, 1H,  $J_{1,2} = 2.8$  Hz, H-2<sub>C</sub>), 5.01 (m, 2H, H-1<sub>B</sub>, 1<sub>E</sub>), 4.96 (m, 2H, H-1<sub>C</sub>, OCH<sub>2</sub>), 4.90-4.81 (m, 5H, H-1<sub>D</sub>, OCH<sub>2</sub>), 4.66-4.41 (m, 7H, OCH<sub>2</sub>), 4.18 (dd, 1H,  $J_{2,3} = 2.9$ ,  $J_{3,4} = 7.4$  Hz, H-3<sub>C</sub>), 4.10 (pt, 1H, H-3<sub>D</sub>), 4.08-3.95 (m, 5H, H-5<sub>E</sub>, 3<sub>E</sub>, 5<sub>C</sub>), 3.89-3.64 (m, 8H, H-6a<sub>D</sub>, 6b<sub>D</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 3<sub>B</sub>, 4<sub>C</sub>, 4<sub>E</sub>, 5<sub>B</sub>), 3.54-3.49 (m, 5H, H-2<sub>E</sub>, 4<sub>D</sub>, OCH<sub>3</sub>), 3.45 (m, 1H, H-5<sub>D</sub>), 3.33 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.4$  Hz, H-4<sub>B</sub>), 3.27 (m, 1H, H-2<sub>D</sub>), 2.26 (bs, 1H, OH), 2.17 (s, 6H, C(O)CH<sub>3</sub>), 1.99 (bs, 1H, OH), 1.39 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>C</sub>), 0.95 (d, 3H,  $J_{5,6} = 6.1$  Hz, H-6<sub>B</sub>);  $^{13}\text{C}$  NMR  $\delta$  171.5, 170.4, 166.1 (3C, CO), 139.1-127.8 (Ph), 100.9 (C-1<sub>D</sub>), 99.7 (2C, C-1<sub>B</sub>\*, 1<sub>C</sub>), 99.2 (bs, C-1<sub>E</sub>), 85.0 (C-3<sub>D</sub>), 82.1 (C-3<sub>E</sub>), 81.3 (bs, C-3<sub>E</sub>), 80.1 (C-4<sub>B</sub>), 78.0, 77.8 (4C, C-3<sub>C</sub>, 4<sub>C</sub>, 3<sub>B</sub>, 4<sub>E</sub>), 76.0 (OCH<sub>2</sub>), 75.6 (C-5<sub>D</sub>), 75.3, 75.2, 74.4, 73.4 (4C, OCH<sub>2</sub>), 72.3 (C-2<sub>C</sub>), 72.1 (C-5<sub>C</sub>\*), 71.3 (C-4<sub>D</sub>), 71.2 (OCH<sub>2</sub>), 69.2 (C-5<sub>B</sub>), 69.0 (C-5<sub>E</sub>, 2<sub>B</sub>), 68.4 (C-6<sub>E</sub>), 63.2 (C-6<sub>D</sub>), 57.4 (2C, C-2<sub>D</sub>, OCH<sub>3</sub>), 23.9, 21.0 (2C, C(O)CH<sub>3</sub>), 19.1 (C-6<sub>C</sub>), 18.0 (C-6<sub>B</sub>). FAB-MS for C<sub>78</sub>H<sub>89</sub>NO<sub>21</sub> (M, 1375.59)  $m/z$  1398.6  $[\text{M}+\text{Na}]^+$ . Anal. Calcd for C<sub>78</sub>H<sub>89</sub>NO<sub>21</sub>: C, 68.06; H, 6.52; N, 1.02%. Found: C, 68.10; H, 6.62; N, 0.98%.

**Methyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (103).** 1% methanolic sodium methoxide (255  $\mu$ L) was added to a suspension of diol 136 (68 mg, 49  $\mu$ mol) in MeOH (2 mL) and the mixture was heated overnight at 55°C. TLC (solvent A, 19:1) showed that the starting material had been converted to a more polar product. Neutralisation with Dowex X8 ( $H^+$ ), evaporation of the volatiles, and column chromatography (solvent A, 24:1) gave tetraol 137 (52 mg, 85%). The latter (48 mg, 39  $\mu$ mol) was dissolved in a mixture of ethanol (5 mL) and ethyl acetate (2 mL) containing 1N aq HCl (50  $\mu$ L). Palladium on charcoal (50 mg) was added and the suspension was stirred under a hydrogen atmosphere overnight. TLC (solvent E, 4:1:2) showed the presence of a single product. Filtration of the catalyst and reverse phase chromatography, followed by RP-HPLC purification and freeze-drying gave pure 103 (19 mg, 71%). Rt: 9.35 min (solvent F, 100:0  $\rightarrow$  80:20 over 20 min);  $[\alpha]_D +12.5$  (c 0.8, methanol);  $^1H$  NMR  $\delta$  5.09 (d, 1H,  $J_{1,2} = 3.7$  Hz, H-1<sub>E</sub>), 4.89 (bs, 1H, H-1<sub>B</sub>), 4.71 (d, 1H,  $J_{1,2} = 1.1$  Hz, H-1<sub>C</sub>), 4.39 (d, 1H,  $J_{1,2} = 8.6$  Hz, H-1<sub>D</sub>), 4.08 (dq, 1H,  $J_{4,5} = 9.3$  Hz, H-5<sub>C</sub>), 3.96 (dd, 1H,  $J_{1,2} = 1.4$ ,  $J_{2,3} = 3.2$  Hz, H-2<sub>B</sub>), 3.88-3.80 (m, 4H, H-2<sub>C</sub>, 3<sub>C</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 5<sub>D</sub>), 3.77-3.62 (m, 6H, H-6a<sub>D</sub>, 6b<sub>D</sub>, 3<sub>B</sub>, 5<sub>B</sub>, 2<sub>D</sub>, 4<sub>C</sub>), 3.59 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.4$  Hz, H-3<sub>E</sub>), 3.50 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.4$  Hz, H-3<sub>E</sub>), 3.50 (pt, 1H,  $J_{3,4} = J_{4,5} = 8.7$  Hz, H-3<sub>D</sub>), 3.47-3.34 (m, 8H, H-2<sub>E</sub>, 4<sub>E</sub>, 4<sub>B</sub>, 4<sub>D</sub>, 5<sub>E</sub>, OCH<sub>3</sub>), 1.98 (s, 3H, C(O)CH<sub>3</sub>), 1.27 (d, 3H,  $J_{5,6} = 6.3$  Hz, H-6<sub>C</sub>), 1.21 (d, 3H,  $J_{5,6} = 6.3$  Hz, H-6<sub>B</sub>);  $^{13}C$  NMR  $\delta$  174.5 (CO), 103.2 (bs, C-1<sub>B</sub>,  $J_{CH} = 172$  Hz), 101.8 (C-1<sub>D</sub>,  $J_{CH} = 160$  Hz), 101.5 (C-1<sub>C</sub>,  $J_{CH} = 170$  Hz), 98.0 (C-1<sub>E</sub>,  $J_{CH} = 170$  Hz), 82.2 (C-3<sub>D</sub>), 79.1 (bs, C-3<sub>C</sub>), 76.6 (bs, C-4<sub>C</sub>), 76.4 (C-4<sub>B</sub>\*), 72.9 (C-3<sub>E</sub>), 72.3, 72.2 (2C, C-4<sub>D</sub>, C-5<sub>D</sub>), 71.87 (C-2<sub>E</sub>), 71.1 (bs, C-2<sub>C</sub>), 70.6 (2C, C-2<sub>B</sub>, 3<sub>B</sub>), 69.7, 69.6 (2C, C-5<sub>E</sub>, 5<sub>B</sub>), 69.2, 68.9 (2C, C-6<sub>D</sub>, 6<sub>E</sub>), 57.4 (OCH<sub>3</sub>), 55.4 (C-2<sub>D</sub>), 22.6 (C(O)CH<sub>3</sub>), 18.0 (C-6<sub>C</sub>), 17.0 (C-6<sub>B</sub>). HRMS (MALDI) Calcd for C<sub>27</sub>H<sub>47</sub>NO<sub>19</sub>Na: 712.2635. Found: 712.2635.

**B- Synthesis of a pentasaccharide building block of the O-specific polysaccharide of *Shigella flexneri* serotype 2a : DAB(E)C**

**Dodecyl 3,4,6-tri-O-acetyl-2-deoxy-1-thio-2-trichloroacetamido- $\beta$ -D-glucopyranoside (205).** A mixture of the peracetylated 204(G. Blatter, J.-M. Beau, J.-C. Jacquinet, *Carbohydr. Res.* 1994, 260, 189-202) (6.2 g, 12.5 mmol) and dodecanthiol (2.5 mL, 94 mmol), 4Å molecular sieves and dry 1,2-DCE (90 mL) was stirred for 1 h, then cooled to 0°C. BF<sub>3</sub>.Et<sub>2</sub>O (1.57 mL, 12.5 mmol) was added. The stirred mixture was allowed to reach rt in 2h30. Et<sub>3</sub>N was added until neutral pH and the mixture filtered. After evaporation, the residue was eluted from a column of silica gel with 2:1 cyclohexane-EtOAc to give 205 as a white solid (7.5 g, 93 %);  $[\alpha]_D -20^\circ$  (c 1, CHCl<sub>3</sub>).  $^1H$  NMR:  $\delta$  6.82 (d, 1H,  $J_{2,NH} = 9.2$  Hz, NH), 5.31 (dd, 1H,  $J_{2,3} = 9.9$ ,  $J_{3,4} = 9.6$  Hz, H-3), 5.15 (dd, 1H,  $J_{4,5} = 9.6$  Hz, H-4), 4.68 (d, 1H,  $J_{1,2} = 10.3$  Hz, H-1), 4.28 (dd, 1H,  $J_{5,6a} = 5.0$ ,  $J_{6a,6b} = 12.3$  Hz, H-6a), 4.17 (dd, 1H,  $J_{5,6b} = 2.3$  Hz, H-6b), 4.11 (dd, 1H, H-2), 3.75 (m,

1H, H-5), 2.70 (m, 2H, SCH<sub>2</sub>), 2.10, 2.05, 2.04 (3s, 9H, OAc), 1.65-1.20 (m, 20H, (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>), 0.90 (t, 3H, (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>). <sup>13</sup>C NMR: δ 171.0, 170.7, 169.3 (C=O), 161.9 (C=OCCl<sub>3</sub>), 92.3 (CCl<sub>3</sub>), 84.2 (C-1), 76.5 (C-5), 73.4 (C-3), 68.6 (C-4), 62.6 (C-6), 55.2 (C-2), 32.3, 30.6, 30.0-29.1, 14.5 (SCH<sub>2</sub>(CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>), 21.1, 21.0, 20.9 (OAc). FAB-MS for C<sub>26</sub>H<sub>42</sub>Cl<sub>3</sub>NO<sub>8</sub>S (M, 635.0) *m/z* 658.1 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>26</sub>H<sub>42</sub>Cl<sub>3</sub>NO<sub>8</sub>S: C, 49.17; H: 6.67; N, 2.21%. Found: C, 49.16; H, 6.71; N, 2.13%.

**Dodecyl 2-deoxy-4,6-*O*-isopropylidene-1-thio-2-trichloroacetamido-β-D-glucopyranoside (207).** A mixture of **205** (5.0 g, 7.87 mmol) in MeOH (15 mL) was deacetylated by catalytic MeONa overnight. The solution was neutralized by IR 120 (H<sup>+</sup>) and filtered. After concentration in vacuo, the residue **206** was treated by 2,2-dimethoxypropane (70 mL) and APTS (148 mg, 0.94 mmol) in DMF (20 mL). After stirring overnight, the mixture was neutralized with Et<sub>3</sub>N and concentrated. The residue was eluted from a column of silica gel with 3:1 cyclohexane-EtOAc to give **207** as a white solid (3.45 g, 80 %); [α]<sub>D</sub> -35° (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR: δ 6.92 (d, 1H, *J*<sub>2,NH</sub> = 8.0 Hz, NH), 4.77 (d, 1H, *J*<sub>1,2</sub> = 10.4 Hz, H-1), 3.98 (m, 1H, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = 9.2 Hz, H-3), 3.88 (dd, 1H, *J*<sub>5,6a</sub> = 5.4, *J*<sub>6a,6b</sub> = 10.8 Hz, H-6a), 3.70 (dd, 1H, *J*<sub>5,6b</sub> = 0.5 Hz, H-6b), 3.63 (m, 1H, H-2), 3.53 (pt, 1H, *J*<sub>4,5</sub> = 9.2 Hz, H-4), 3.29 (m, 1H, H-5), 2.98 (s, 1H, OH), 2.60 (m, 2H, SCH<sub>2</sub>), 1.60-1.10 (m, 20H, (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>), 1.45, 1.35 (2s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 0.80 (t, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR: δ 162.5 (C=OCCl<sub>3</sub>), 100.3 (C(CH<sub>3</sub>)<sub>2</sub>), 92.8 (CCl<sub>3</sub>), 84.0 (C-1), 74.6 (C-4), 72.3 (C-3), 71.7 (C-5), 62.2 (C-6), 58.3 (C-2), 29.3, 19.5 (C(CH<sub>3</sub>)<sub>2</sub>), 32.3, 30.8, 30.1-29.5, 29.1, 14.5 (SCH<sub>2</sub>(CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>). FAB-MS for C<sub>23</sub>H<sub>40</sub>Cl<sub>3</sub>NO<sub>5</sub>S (M, 548.9) *m/z* 572.2 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>23</sub>H<sub>40</sub>Cl<sub>3</sub>NO<sub>5</sub>S: C, 50.32; H, 7.34; N, 2.55%. Found: C, 50.30; H, 7.40; N, 2.36%.

**Dodecyl 3-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-1-thio-2-trichloroacetamido-β-D-glucopyranoside (208).** A mixture of **207** (1.07 g, 1.94 mmol) in pyridine (10 mL) was cooled to 0°C. Ac<sub>2</sub>O (5 mL) was added and the solution was allowed to reach rt in 2 h. The mixture was then concentrated and pyridine was coevaporated with toluene. The residue was eluted from a column of silica gel with 6:1 cyclohexane-EtOAc with 0.2% of Et<sub>3</sub>N to give **208** as a white solid (1.12 g, 97 %); [α]<sub>D</sub> -62° (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR: δ 7.51 (d, 1H, *J*<sub>2,NH</sub> = 9.7 Hz, NH), 5.40 (dd, 1H, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = 10.0 Hz, H-3), 4.62 (d, 1H, *J*<sub>1,2</sub> = 10.4 Hz, H-1), 4.20 (m, 1H, H-2), 4.01 (dd, 1H, *J*<sub>5,6a</sub> = 5.2, *J*<sub>6a,6b</sub> = 10.7 Hz, H-6a), 3.84 (dd, 1H, *J*<sub>4,5</sub> = 9.7 Hz, H-4), 3.70 (m, 2H, H-5, H-6b), 2.68 (m, 2H, SCH<sub>2</sub>), 2.09 (s, 3H, OAc), 1.60-1.20 (m, 20H, (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>), 1.52, 1.38 (2 s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 0.90 (t, 3H, SCH<sub>2</sub>(CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>). <sup>13</sup>C NMR: δ 171.4 (C=O), 161.8 (C=OCCl<sub>3</sub>), 99.5 (C(CH<sub>3</sub>)<sub>2</sub>), 92.3 (CCl<sub>3</sub>), 84.6 (C-1), 73.6 (C-3), 72.0 (C-4), 71.9 (C-5), 62.2 (C-6), 55.0 (C-2), 29.1, 19.3 (C(CH<sub>3</sub>)<sub>2</sub>), 32.3, 30.7, 30.0-29.0, 14.5 (SCH<sub>2</sub>(CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>). FAB-MS for C<sub>25</sub>H<sub>42</sub>Cl<sub>3</sub>NO<sub>6</sub>S (M, 591.0) *m/z* 614.1 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>25</sub>H<sub>42</sub>Cl<sub>3</sub>NO<sub>6</sub>S: C, 50.80; H, 7.16; N, 2.37%. Found: C, 50.67; H, 7.32; N, 2.24%.

**Allyl 3,4-di-O-benzyl-2-O-levulinoyl- $\alpha$ -L-rhamnopyranoside (210).**

DCC (5.76 g, 28.0 mmol), levulinic acid (2.65 g, 22.8 mmol) and DMAP (115 mg) were added to a solution of alcohol **209** (1.65 g, 4.29 mmol) in THF (70 mL). The suspension was stirred at rt overnight. Et<sub>2</sub>O was added and solids were filtered. The filtrate was concentrated, and the residue was purified twice from a column of silica gel, eluting first with 99.5:0.5 to 98:2 DCM-EtOAc, then with 9:1 cyclohexane-acetone. The target **210** (2.00g 97%) as a colourless oil slightly contaminated by a less polar product.. <sup>1</sup>H NMR:  $\delta$  7.40-7.30 (m, 10H, Ph), 5.90 (m, 1H, All), 5.40 (dq, 1H,  $J_{1,2} = 1.8$ ,  $J_{2,3} = 3.4$  Hz, H-2), 5.28 (m, 1H, All), 5.20 (m, 1H, All), 4.93 (d, 1H,  $J = 10.8$  Hz, CH<sub>2</sub>Ph), 4.78 (d, 1H,  $J_{1,2} = 1.6$  Hz, H-1), 4.78 (d, 1H,  $J = 11.2$  Hz, CH<sub>2</sub>Ph), 4.63 (d, 1H, CH<sub>2</sub>Ph), 4.51 (d, 1H, CH<sub>2</sub>Ph), 4.17 (m, 2H, All, H-3), 3.78 (dq, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.2$  Hz, H-5), 3.43 (pt, 1H,  $J_{3,4} = 9.5$  Hz, H-4), 2.80 (m, 4H, Lev), 2.19 (s, 3H, Ac), 1.37 (d, 3H, H-6). <sup>13</sup>C NMR:  $\delta$  124.0-125.1 (Ph), 118.0 (All), 97.0 (C-1), 80.2 (C-4), 78.5 (C-3), 75.2 (CH<sub>2</sub>Ph), 72.0 (CH<sub>2</sub>Ph), 70.2 (C-2), 68.5 (All), 68.3 (C-5), 38.5 (Lev), 31.5 (Ac), 28.5 (Lev), 20.1 (C-6). Anal. Calcd for C<sub>25</sub>H<sub>30</sub>O<sub>7</sub>: C, 69.69; H, 7.10. Found: C, 69.61; H, 7.10.

**3,4-Di-O-benzyl-2-O-levulinoyl- $\alpha$ -L-rhamnopyranose (211).**

1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (25 mg, 20  $\mu$ mol) was dissolved THF and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **210** (1.4 g, 3.12 mmol) in THF was degassed and added. The mixture was stirred at rt overnight, then concentrated to dryness. The residue was dissolved in a solution of I<sub>2</sub> (1.37 g, 5.4 mmol) in 30 mL of THF/H<sub>2</sub>O (15:4). The mixture was stirred at rt for 1 h, and THF was evaporated. The resulting suspension was taken up in DCM, washed twice with water, satd aq NaHSO<sub>3</sub>, water, satd aq NaHCO<sub>3</sub>, water and satd aq NaCl, successively. The organic layer was dried and concentrated. The residue was eluted from a column of silica gel with 7:3 to 6:4 Cyclohexane-EtOAc to give the corresponding hemiacetal **211** (1.3 g, 93 %). <sup>1</sup>H NMR:  $\delta$  7.40-7.30 (m, 10H, Ph), 5.40 (dq, 1H,  $J_{1,2} = 1.8$ ,  $J_{2,3} = 3.4$  Hz, H-2), 4.93 (d, 1H,  $J = 10.8$  Hz, CH<sub>2</sub>Ph), 4.78 (d, 1H,  $J_{1,2} = 1.6$  Hz, H-1), 4.78 (d, 1H,  $J = 11.2$  Hz, CH<sub>2</sub>Ph), 4.63 (d, 1H, CH<sub>2</sub>Ph), 4.51 (d, 1H, CH<sub>2</sub>Ph), 3.99 (m, 1H,  $J_{3,4} = 9.5$  Hz, H-3), 3.78 (dq, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.2$  Hz, H-5), 3.43 (pt, 1H, H-4), 2.80 (m, 4H, Lev), 2.19 (s, 3H, Ac), 1.37 (d, 3H, H-6). Anal. Calcd for C<sub>28</sub>H<sub>34</sub>O<sub>7</sub>: C, 67.86; H, 6.83. Found: C, 67.94; H, 6.87.

**3,4-Di-O-benzyl-2-O-levulinoyl- $\alpha$ -L-rhamnopyranosyl**

**trichloroacetimidate (212).** Trichloroacetonitrile (1.3 mL, 13 mmol) and DBU (51  $\mu$ L, 0.3 mmol) were added to a solution of the residue **211** (1.0 g, 2.3 mmol) in anhydrous DCM (6 mL) at 0°C. After 2 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 3:1 cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give **212** as a white foam (1.0 g, 95 %); <sup>1</sup>H NMR:  $\delta$  8.67 (s, 1H, NH), 7.40-7.30 (m, 10H, Ph), 6.19 (d, 1H,  $J_{1,2}$

= 1.9 Hz, H-1), 5.48 (dd, 1H,  $J_{1,2} = 2.0$ ,  $J_{2,3} = 3.3$  Hz, H-2), 4.95 (d, 1H, CH<sub>2</sub>Ph), 4.73 (d, 1H, CH<sub>2</sub>Ph), 4.66 (d, 1H, CH<sub>2</sub>Ph), 4.58 (d, 1H, CH<sub>2</sub>Ph), 4.51 (d, 1H, CH<sub>2</sub>Ph), 4.00 (dd, 1H,  $J_{3,4} = 9.5$  Hz, H-3), 3.95 (dq, 1H,  $J_{4,5} = 9.6$ ,  $J_{5,6} = 6.3$  Hz, H-5), 3.52 (pt, 1H, H-4), 2.80 (m, 4H, Lev), 2.20 (s, 3H, Ac), 1.36 (d, 3H, H-6). Anal. Calcd for C<sub>27</sub>H<sub>30</sub>Cl<sub>3</sub>NO<sub>7</sub>·0.5H<sub>2</sub>O: C, 54.42; H, 5.24; N, 2.35. Found: C, 54.06; H, 5.06; 2.05.

**Allyl (2-*O*-levulinoyl-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (215).** A mixture of alcohol **214** (F. Segat, L. A. Mulard, *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) (300 mg, 0.36 mmol) and imidate **212** (320 mg, 0.54 mmol) in anhydrous Et<sub>2</sub>O (20 mL) was stirred for 15 min under dry Ar. After cooling at -75°C, Me<sub>3</sub>SiOTf (13  $\mu$ L, 70  $\mu$ mol) was added dropwise and the mixture was stirred 3 h. Et<sub>3</sub>N (60  $\mu$ L) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 9:1 cyclohexane-EtOAc to give **215** (440 mg, 92 %) as a colourless foam. <sup>1</sup>H NMR:  $\delta$  8.10-7.10 (m, 35H, Ph), 5.95 (m, 1H, All), 5.73 (dd, 1H,  $J_{1,2} = 2.2$ ,  $J_{2,3} = 2.3$  Hz, H-2<sub>B</sub>), 5.43 (dd, 1H,  $J_{1,2} = 2.0$ ,  $J_{2,3} = 3.0$  Hz, H-2<sub>C</sub>), 5.30 (m, 2H, All), 5.08 (d, 1H,  $J_{1,2} = 3.2$  Hz, H-1<sub>E</sub>), 5.03 (d, 1H,  $J_{1,2} = 1.7$  Hz, H-1<sub>B</sub>), 4.97 (d, 1H,  $J_{1,2} = 1.9$  Hz, H-1<sub>C</sub>), 4.30-5.00 (m, 12H, CH<sub>2</sub>Ph), 4.20 (m, 2H, All, H-3<sub>C</sub>), 4.05 (m, 3H, All, H-3<sub>E</sub>, 5<sub>E</sub>), 3.98 (m, 1H, H-6<sub>A</sub>), 3.81 (m, 5H, H-3<sub>B</sub>, 4<sub>C</sub>, 4<sub>E</sub>, 5<sub>C</sub>, 6<sub>E</sub>), 3.69 (dq, 1H,  $J_{4,5} = 9.3$ ,  $J_{5,6} = 6.0$  Hz, H-5<sub>B</sub>), 3.52 (dd, 1H,  $J_{2,3} = 9.7$  Hz, H-2<sub>E</sub>), 3.29 (dd, 1H,  $J_{3,4} = J_{4,5} = 9.4$  Hz, H-4<sub>B</sub>), 2.71 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>), 2.15 (s, 3H, Ac), 1.40 (d, 3H, H-6<sub>C</sub>), 1.01 (d, 3H, H-6<sub>B</sub>).

**Allyl (3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (216).** The trisaccharide **215** (200 mg, 0.16 mmol) was treated with 0.4 mL of a solution 1 M of hydrazine (100 mg) diluted in a mixture of pyridine (1.6 mL) and acetic acid (0.4 mL) at rt. The solution was stirred during 20 min. Acetone (1.2 mL) was added and the solution was concentrated. The residue was eluted from a column of silica gel with 98.5:1.5 DCM-EtOAc to give **216** (174 mg) as a foam. Although, contaminated with hydrazine salts, the <sup>1</sup>H NMR spectrum showed that compound **216** had NMR data identical to that of a reference compound. (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074)

**Allyl (2-*O*-levulinoyl-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (217).** Triflic acid (3.5  $\mu$ L, 40  $\mu$ mol) was added to a mixture of the donor **212** (88 mg, 265  $\mu$ mol), the acceptor **216** (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074) (197 mg, 176  $\mu$ mol), and 4Å molecular sieves in dry DCM (2.5 mL) kept under stirring at -30°C. The suspension was stirred for 1 h at this temperature, then at rt for 2 h. More **212** (40 mg, 120  $\mu$ mol) was added and the mixture was kept at 4°C for 40 h. After

addition of more triflic acid (1  $\mu$ L, 11  $\mu$ mol) and stirring for 2 h at rt, Et<sub>3</sub>N was added to the reaction mixture. Filtration through a pad of Celite, and evaporation of the volatiles resulted in a oily residue which was purified by flash chromatography with 7:3 cyclohexane-EtOAc to give **217** (123 mg, 54%).

<sup>1</sup>H NMR:  $\delta$  8.10-7.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.61 (bs, 1H, H-2<sub>A</sub>), 5.48 (bs, 1H, H-2<sub>C</sub>), 5.34 (m, 2H, All), 4.97 (bs, 2H, H-1<sub>B</sub>, 1<sub>E</sub>), 5.10 (bs, 1H, H-1<sub>C</sub>), 5.02 (bs, 1H, H-1<sub>A</sub>), 5.06-4.37 (m, 16H, CH<sub>2</sub>Ph), 4.45 (bs, 1H, H-2<sub>B</sub>), 4.28-3.83 (m, 8H, H-3<sub>E</sub>, 5<sub>E</sub>, 3<sub>A</sub>, 5<sub>A</sub>, 3<sub>C</sub>, 5<sub>C</sub>), 3.83 (m, 3H, H-6<sub>aE</sub>, 6<sub>bE</sub>, 4<sub>C</sub>), 3.80 (m, 1H, H-4<sub>E</sub>), 3.72 (dd, 1H, H-3<sub>B</sub>), 3.66 (m, 1H, H-5<sub>B</sub>), 3.57 (dd, 1H, H-2<sub>E</sub>), 3.51 (dd, 1H, H-4<sub>A</sub>), 3.39 (dd, 1H, H-4<sub>B</sub>), 2.66 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>), 2.13 (s, 3H, CH<sub>3</sub>), 1.45 (2d, 6H, H-6<sub>A</sub>, 6<sub>C</sub>), 1.07 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR:  $\delta$  206.4, 172.1, 166.2 (3C, C=O), 139.2-127.6 (Ph), 118.1 (All), 101.4 (C-1<sub>B</sub>), 99.7 (C-1<sub>A</sub>), 98.3 (C-1<sub>E</sub>), 96.5 (C-1<sub>C</sub>), 82.3 (C-3<sub>E</sub>), 81.5 (C-2<sub>E</sub>), 80.5 (C-3<sub>C</sub>), 80.2 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 79.3 (C-3<sub>B</sub>), 78.6 (C-3<sub>A</sub>), 78.0 (2C, C-4<sub>C</sub>, 4<sub>E</sub>), 76.0, 75.8, 75.6 (3C, CH<sub>2</sub>Ph), 75.2 (C-2<sub>B</sub>), 75.0, 74.4, 73.4 (3C, CH<sub>2</sub>Ph), 72.9 (C-2<sub>C</sub>), 72.0 (CH<sub>2</sub>Ph), 71.8 (C-5<sub>E</sub>), 71.1 (CH<sub>2</sub>Ph), 69.8 (C-2<sub>A</sub>), 69.3 (C-5<sub>B</sub>), 68.9, 68.8 (All, C-6<sub>E</sub>), 68.7 (C-5<sub>A</sub>), 68.0 (C-5<sub>C</sub>), 38.5, 28.5 (2C, CH<sub>2</sub>CO), 30.2 (CH<sub>3</sub>), 19.2, 18.8, 18.2 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>).

**Allyl (3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-O-benzoyl- $\alpha$ -L-rhamnopyranoside (218).** The tetrasaccharide **217** (121 mg, 0.09 mmol) was treated with 235  $\mu$ L of a 1 M solution of hydrazine hydrate (100 mg) in a mixture of pyridine (1.6 mL) and acetic acid (0.4 mL) at rt. The solution was stirred during 15 min. Acetone (3 mL) was added and the solution was concentrated. The residue was eluted from a column of silica gel with 9:1 cyclohexane-acetone to give alcohol **218** (70 mg). Compound **218** had NMR data identical to that of a reference compound. (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074)

**Allyl (3-O-acetyl-4,6-O-isopropylidene-2-trichloroacetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-O-benzoyl- $\alpha$ -L-rhamnopyranoside (219).** A mixture of the donor **208** (294 mg, 357  $\mu$ mol), the acceptor **218** (F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060-1074) (313 mg, 211  $\mu$ mol), and 4Å molecular sieves in dry DCM (4 mL) was stirred for 1.5 h then cooled to -15°C. NIS (94 mg, 0.42 mmol) and triflic acid (8  $\mu$ L, 0.1 mmol) were successively added. The stirred mixture was allowed to reach 0°C in 1.5 h. Et<sub>3</sub>N (25  $\mu$ L) was added and the mixture filtered. After evaporation, the residue was eluted from a column of silica gel with 6:1 cyclohexane-EtOAc and 0.5 % of Et<sub>3</sub>N to give **219** as a white foam (232 mg, 58 %); [ $\alpha$ ]<sub>D</sub> -2° (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR:  $\delta$  7.00-8.00 (m, 45H, Ph), 6.81 (d, 1H, *J*<sub>2,NH</sub> = 9.0 Hz, NH<sub>D</sub>), 5.82 (m, 1H, All), 5.30 (dd, 1H, *J*<sub>1,2</sub> = 1.0, *J*<sub>2,3</sub> = 3.0 Hz, H-2<sub>C</sub>), 5.10-5.23 (m, 2H, All),

4.96 (bs, 1H, H-1<sub>A</sub>), 4.91 (d, 1H,  $J_{1,2} = 3.1$  Hz, H-1<sub>E</sub>), 4.87 (d, 1H,  $J_{1,2} = 1.6$  Hz, H-1<sub>B</sub>), 4.84 (bs, 1H, H-1<sub>C</sub>), 4.79 (dd, 1H,  $J_{2,3} = J_{3,4} = 10.0$  Hz, H-3<sub>D</sub>), 4.35 (d, 1H, H-1<sub>D</sub>), 4.34 (dd, 1H, H-2<sub>B</sub>), 4.20-4.80 (m, 16H, CH<sub>2</sub>Ph), 4.00 (dd, 1H, H-2<sub>A</sub>), 3.90 (dd, 1H, H-2<sub>D</sub>), 2.90-4.10 (m, 22H, All, H-2<sub>E</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>D</sub>, 5<sub>E</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>), 1.93 (s, 3H, OAc), 1.2-0.9 (m, 15H, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR: δ 170.7, 165.5, 161.7 (C=O), 138.4-117.3 (Ph, All), 101.7 (C-1<sub>D</sub>), 100.8 (C-1<sub>B</sub>), 100.6 (C-1<sub>A</sub>), 99.5 (C(CH<sub>3</sub>)<sub>2</sub>), 97.9 (C-1<sub>E</sub>), 95.7 (C-1<sub>C</sub>), 92.0 (CCl<sub>3</sub>), 82.2, 81.7, 81.6, 80.3, 79.9, 78.8, 77.9, 77.9, 76.6, 76.0, 75.8, 75.4, 75.1, 74.7, 74.3, 74.1, 73.3, 72.8, 72.6, 71.9, 71.5, 70.8, 69.0, 68.8, 68.5, 68.0, 67.8, 62.0, 56.7 (C-2<sub>D</sub>), 28.6 (C(CH<sub>3</sub>)<sub>2</sub>), 21.3 (OAc), 19.4 (C(CH<sub>3</sub>)<sub>2</sub>), 19.0, 18.5, 18.4 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>).

**Allyl (2-acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (201).** A mixture of **219** (144 mg, 0.06 mmol), Bu<sub>3</sub>SnH (0.1 mL, 0.37 mmol) and AIBN (10 mg) in dry toluene (3 mL) was stirred for 1 h at rt under a stream of dry Ar, then was heated for 1.5 h at 90°C, cooled and concentrated. The residue was eluted from a column of silica gel with 2:1 cyclohexane-EtOAc and 0.2 % of Et<sub>3</sub>N to give **201** (100 mg, 74 %). <sup>1</sup>H NMR: δ 6.95-8.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.46 (d, 1H,  $J_{2,NH} = 8.0$  Hz, NH<sub>D</sub>), 5.29 (dd, 1H,  $J_{1,2} = 1.0$ ,  $J_{2,3} = 3.0$  Hz, H-2<sub>C</sub>), 5.11-5.25 (m, 2H, All), 5.00 (bs, 1H, H-1<sub>A</sub>), 4.90 (d, 1H,  $J_{1,2} = 3.1$  Hz, H-1<sub>E</sub>), 4.85 (d, 1H,  $J_{1,2} = 1.6$  Hz, H-1<sub>B</sub>), 4.83 (bs, 1H, H-1<sub>C</sub>), 4.70 (dd, 1H,  $J_{2,3} = J_{3,4} = 10.0$  Hz, H-3<sub>D</sub>), 4.44 (d, 1H, H-1<sub>D</sub>), 4.34 (dd, 1H, H-2<sub>B</sub>), 4.20-4.80 (m, 16H, CH<sub>2</sub>Ph), 4.02 (dd, 1H, H-2<sub>A</sub>), 3.37 (dd, 1H, H-2<sub>E</sub>), 2.90-4.10 (m, 21H, All, H-2<sub>D</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>D</sub>, 5<sub>E</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>), 1.92 (s, 3H, OAc), 1.57 (s, 3H, AcNH), 1.27-0.90 (m, 15H, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR: δ 171.3, 170.3, 166.2 (C=O), 138.7-117.9 (Ph, All), 103.9 (C-1<sub>D</sub>), 101.5 (C-1<sub>B</sub>), 101.4 (C-1<sub>A</sub>), 99.9 (C(CH<sub>3</sub>)<sub>2</sub>), 98.5 (C-1<sub>E</sub>), 96.3 (C-1<sub>C</sub>), 82.1, 81.7, 81.6, 80.3, 80.1, 78.8, 78.1, 77.8, 76.0, 75.8, 75.3, 75.1, 74.7, 74.2, 73.6, 73.3, 72.7, 71.9, 71.4, 70.8, 69.0, 68.8, 68.7, 68.4, 68.1, 67.8, 62.1, 55.0 (C-2<sub>D</sub>), 30.0 (C(CH<sub>3</sub>)<sub>2</sub>), 23.5 (AcNH), 21.6 (OAc), 19.2 (C(CH<sub>3</sub>)<sub>2</sub>), 19.0, 18.3, 18.2 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). FAB-MS for C<sub>103</sub>H<sub>117</sub>NO<sub>25</sub> (M, 1769.0)  $m/z$  1791.9 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>103</sub>H<sub>117</sub>NO<sub>25</sub>: C, 69.93; H, 6.67; N, 0.79. Found: C, 69.77; H, 6.84; N, 0.72.

**(2-Acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (203).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (50 mg, 58 μmol) was dissolved THF (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to



yellow. The solution was then degassed again in an argon stream. A solution of **201** (1.8 g, 1.02 mmol) in THF (20 mL) was degassed and added. The mixture was stirred at rt overnight then concentrated to dryness. The residue was dissolved in acetone (9 mL), then water (2 mL), mercuric chloride (236 mg) and mercuric oxide (200 mg) were added successively. The mixture protected from light was stirred at rt for 2 h and acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aq KI, water and satd aq NaCl, dried and concentrated. The residue was eluted from a column of silica gel with 3:2 Cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give the corresponding hemiacetal **220**. Trichloroacetonitrile (2.4 mL) and DBU (72  $\mu$ L) were added to a solution of the residue in anhydrous DCM (24 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 3:2 cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give **203** as a colourless oil (1.58 g, 82 %); [ $\alpha$ ]<sub>D</sub> +2° (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  8.62 (s, 1H, C=NH), 6.95-8.00 (m, 45H, Ph), 6.24 (d, 1H,  $J_{1,2}$  = 2.6 Hz, H-1<sub>C</sub>), 5.48 (dd, 1H,  $J_{2,3}$  = 3.0 Hz, H-2<sub>C</sub>), 5.41 (d, 1H,  $J_{2,NH}$  = 8.4 Hz, NH<sub>D</sub>), 4.99 (bs, 1H, H-1<sub>A</sub>), 4.92 (d, 1H,  $J_{1,2}$  = 3.2 Hz, H-1<sub>E</sub>), 4.88 (d, 1H,  $J_{1,2}$  = 1.6 Hz, H-1<sub>B</sub>), 4.69 (dd, 1H,  $J_{2,3}$  =  $J_{3,4}$  = 10.0 Hz, H-3<sub>D</sub>), 4.44 (d, 1H, H-1<sub>D</sub>), 4.34 (dd, 1H, H-2<sub>B</sub>), 4.20-4.80 (m, 16H, CH<sub>2</sub>Ph), 4.02 (dd, 1H, H-2<sub>A</sub>), 3.38 (dd, 1H, H-2<sub>E</sub>), 2.90-4.10 (m, 19H, H-2<sub>D</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>D</sub>, 5<sub>E</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>), 1.95 (s, 3H, OAc), 1.55 (s, 3H, AcNH), 1.30-0.85 (m, 15H, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR:  $\delta$  172.4, 171.4, 166.9 (C=O), 140.2-128.9 (Ph), 104.2 (C-1<sub>D</sub>), 101.4 (2C, C-1<sub>A</sub>, 1<sub>B</sub>), 101.1 (C(CH<sub>3</sub>)<sub>2</sub>), 98.0 (C-1<sub>E</sub>), 94.8 (C-1<sub>C</sub>), 92.4 (CCl<sub>3</sub>), 82.1, 81.5, 80.2, 80.1, 78.6, 78.1, 77.8, 77.6, 76.0, 75.8, 75.5, 75.0, 74.3, 74.2, 73.5 (C-3<sub>D</sub>), 73.4, 71.9, 71.4, 71.0, 70.5, 69.2, 68.8, 68.3, 68.1, 62.1, 54.9 (C-2<sub>D</sub>), 29.3 (C(CH<sub>3</sub>)<sub>2</sub>), 23.4 (AcNH), 21.4 (OAc), 19.2 (C(CH<sub>3</sub>)<sub>2</sub>), 19.0, 18.2, 18.1 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). FAB-MS for C<sub>102</sub>H<sub>113</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>25</sub> (M, 1873.3) *m/z* 1896.3 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>102</sub>H<sub>113</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>25</sub>: C, 65.40; H, 6.08; N, 1.50. Found: C, 65.26; H, 6.02; N, 1.31.

C. Convergent synthesis of the decasaccharide  
D'A'B'(E')C'DAB(E)C as its methyl glycoside

Phenyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl-1-thio- $\alpha$ -L-rhamnopyranoside (**308**). A mixture of alcohol **315** (0.12 g, 0.27 mmol) and imidate **316** (0.245 g, 0.41 mmol) in anhydrous DCM (10 mL) was stirred for 15 min under dry argon. After cooling at 0°C, Me<sub>3</sub>SiOTf (28  $\mu$ L) was added dropwise and the mixture was stirred for 0.5 h. Triethylamine (60  $\mu$ L) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 4:1 cyclohexane-EtOAc to give **308** (227 mg, 97 %) as a colourless foam; [ $\alpha$ ]<sub>D</sub> -63° (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  7.40-7.10 (m, 15H, Ph), 6.73 (d, 1H,  $J_{2,NH}$  = 8.5 Hz, NH<sub>D</sub>), 5.47 (d, 1H,  $J_{1,2}$  = 1.2 Hz, H-1<sub>A</sub>), 5.07 (pt, 1H,  $J_{2,3}$  =  $J_{3,4}$  = 10.0 Hz, H-3<sub>D</sub>), 4.99 (pt, 1H,  $J_{4,5}$  = 10.0 Hz, H-4<sub>D</sub>), 4.80-4.55 (m, 4H, CH<sub>2</sub>Ph), 4.52 (d, 1H,  $J_{1,2}$  = 8.2 Hz, H-1<sub>D</sub>), 4.13-3.95 (m, 2H,  $J_{5,6}$  = 5.3,  $J_{6a,6b}$  = 12.2 Hz, H-6<sub>aD</sub>, 6<sub>bD</sub>), 4.10 (dq,

1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.1$  Hz, H-5<sub>A</sub>), 4.00 (dd, 1H,  $J_{2,3} = 3.0$  Hz, H-2<sub>A</sub>), 3.99 (m, 1H, H-2<sub>D</sub>), 3.77 (dd, 1H,  $J_{3,4} = 9.4$  Hz, H-3<sub>A</sub>), 3.50 (m, 1H, H-5<sub>D</sub>), 3.39 (dd, 1H, H-4<sub>A</sub>), 1.95, 1.93, 1.90 (3s, 9H, OAc), 1.23 (d, 3H, H-6<sub>A</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  171.1, 170.9, 169.6, 162.1 (C=O), 138-127 (Ph), 102.1 (C-1<sub>D</sub>), 92.7 (CCl<sub>3</sub>), 87.4 (C-1<sub>A</sub>), 81.3 (C-4<sub>A</sub>), 80.5 (C-3<sub>A</sub>), 79.1 (C-2<sub>A</sub>), 76.4, 74.1 (2C, CH<sub>2</sub>Ph), 72.4 (C-5<sub>D</sub>), 72.4 (C-3<sub>D</sub>), 69.8 (C-5<sub>A</sub>), 68.7 (C-4<sub>D</sub>), 62.3 (C-6<sub>D</sub>), 56.2 (C-2<sub>D</sub>), 21.0, 20.9, 20.8 (3C, OAc), 18.1 (C-6<sub>A</sub>). FAB-MS for C<sub>40</sub>H<sub>44</sub>Cl<sub>3</sub>NO<sub>12</sub>S (M, 867),  $m/z$  890 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>40</sub>H<sub>44</sub>Cl<sub>3</sub>NO<sub>12</sub>S: C, 55.27; H, 5.10; N, 1.61. Found: C, 55.16; H, 5.18; N, 1.68.

**Allyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranoside (317).** A mixture of alcohol 314 (1.86 g, 4.86 mmol) and imidate 316 (3.85 g, 6.47 mmol) in anhydrous CH<sub>3</sub>CN (80 mL) was stirred for 15 min under dry Ar. After cooling at 0°C, Me<sub>3</sub>SiOTf (46  $\mu$ L) was added dropwise and the mixture was stirred for 0.5 h. Triethylamine (150  $\mu$ L) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 7:3 cyclohexane-EtOAc to give 317 (4.0 g, 99 %) as a white solid;  $[\alpha]_D -3^\circ$  (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  7.32-7.18 (m, 10H, Ph), 6.70 (d, 1H,  $J_{2,NH} = 8.4$  Hz, NH<sub>D</sub>), 5.82-5.78 (m, 1H, All), 5.20-5.05 (m, 2H, All), 5.00 (m, 2H, H-3<sub>D</sub>, 4<sub>D</sub>), 4.75-4.45 (m, 4H, CH<sub>2</sub>Ph), 4.76 (d, 1H,  $J_{1,2} = 1.1$  Hz, H-1<sub>A</sub>), 4.60 (d, 1H,  $J_{1,2} = 8.5$  Hz, H-1<sub>D</sub>), 4.15-4.05 (m, 2H,  $J_{5,6} = 4.8$ ,  $J_{6a,6b} = 12.2$  Hz, H-6a<sub>D</sub>, 6b<sub>D</sub>), 3.98 (m, 1H, H-2<sub>D</sub>), 3.90 (m, 2H, All), 3.86 (dd, 1H,  $J_{2,3} = 3.2$  Hz, H-2<sub>A</sub>), 3.81 (dd, 1H,  $J_{3,4} = 9.5$  Hz, H-3<sub>A</sub>), 3.62 (dq, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.1$  Hz, H-5<sub>A</sub>), 3.50 (m, 1H, H-5<sub>D</sub>), 3.32 (pt, 1H, H-4<sub>A</sub>), 2.02, 1.97, 1.93 (3 s, 9H, OAc), 1.24 (d, 3H, H-6<sub>A</sub>); <sup>13</sup>C NMR:  $\delta$  171.0, 170.9, 169.6, 162.1 (C=O), 138.5-117.1 (Ph, All), 101.8 (C-1<sub>D</sub>), 98.5 (C-1<sub>A</sub>), 92.6 (CCl<sub>3</sub>), 81.4 (C-4<sub>A</sub>), 80.4 (C-3<sub>A</sub>), 77.1 (C-2<sub>A</sub>), 75.9, 74.1 (2C, CH<sub>2</sub>Ph), 72.7 (C-3<sub>D</sub>), 72.5 (C-5<sub>D</sub>), 68.6 (C-4<sub>D</sub>), 68.3 (C-5<sub>A</sub>), 68.1 (All), 62.3 (C-6<sub>D</sub>), 56.1 (C-2<sub>D</sub>), 21.1, 20.9, 20.9 (3C, OAc), 18.2 (C-6<sub>A</sub>). FAB-MS for C<sub>37</sub>H<sub>44</sub>Cl<sub>3</sub>NO<sub>13</sub> (M, 815),  $m/z$  838 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>37</sub>H<sub>44</sub>Cl<sub>3</sub>NO<sub>13</sub>: C, 54.39; H, 5.43; N, 1.71%. Found: C, 54.29; H, 5.45; N, 1.72%.

**(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranose (318).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (120 mg, 140  $\mu$ mol) was dissolved in tetrahydrofuran (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of 317 (1.46 g, 1.75 mmol) in tetrahydrofuran (20 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (27 mL), and water (3 mL) was added. Mercuric bromide (949 mg, 2.63 mmol) and mercuric oxide (761 mg, 3.5 mmol) were added to the mixture, protected from light. The mixture was stirred for 2 h at rt, then concentrated. The residue was taken up in

CH<sub>2</sub>Cl<sub>2</sub> and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (cyclohexane-EtOAc 4:1) to give **318** (1.13 g, 81 %) as a white foam.  $[\alpha]_D +4^\circ$  (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  7.35-7.05 (m, 10H, Ph), 6.74 (d, 1H,  $J_{2,NH} = 8.5$  Hz, NH<sub>D</sub>), 5.10 (d, 1H,  $J_{1,2} = 1.1$  Hz, H-1<sub>A</sub>), 5.02 (m, 2H, H-3<sub>D</sub>, 4<sub>D</sub>), 4.80-4.50 (m, 4H, CH<sub>2</sub>Ph), 4.61 (d, 1H,  $J_{1,2} = 8.5$  Hz, H-1<sub>D</sub>), 4.15-4.08 (m, 2H,  $J_{5,6} = 4.5$ ,  $J_{6a,6b} = 12.3$  Hz, H-6a<sub>D</sub>, 6b<sub>D</sub>), 4.00 (m, 1H, H-2<sub>D</sub>), 3.90 (dd, 1H,  $J_{2,3} = 3.3$ , H-2<sub>A</sub>), 3.86 (dd, 1H,  $J_{3,4} = 9.5$  Hz, H-3<sub>A</sub>), 3.85 (dq, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.2$  Hz, H-5<sub>A</sub>), 3.50 (m, 1H, H-5<sub>D</sub>), 3.30 (pt, 1H, H-4<sub>A</sub>), 2.85 (d, 1H,  $J_{1,OH} = 3.5$  Hz, OH), 2.02, 1.97, 1.94 (3s, 9H, OAc), 1.23 (d, 3H, H-6<sub>A</sub>); <sup>13</sup>C NMR:  $\delta$  171.1, 170.0, 169.6, 162.1 (C=O), 138.5-127.1 (Ph), 101.7 (C-1<sub>D</sub>), 94.1 (C-1<sub>A</sub>), 92.6 (CCl<sub>3</sub>), 81.4 (C-4<sub>A</sub>), 79.9 (C-2<sub>A</sub>), 77.3 (C-3<sub>A</sub>), 75.9, 74.1 (2C, CH<sub>2</sub>Ph), 72.7 (C-3<sub>D</sub>), 72.5 (C-5<sub>D</sub>), 68.6 (C-4<sub>D</sub>), 68.4 (C-5<sub>A</sub>), 62.2 (C-6<sub>D</sub>), 56.1 (C-2<sub>D</sub>), 21.1, 21.0, 20.9 (3C, OAc), 18.3 (C-6<sub>A</sub>). FAB-MS for C<sub>34</sub>H<sub>40</sub>Cl<sub>3</sub>NO<sub>13</sub> (M, 775),  $m/z$  789 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>34</sub>H<sub>40</sub>Cl<sub>3</sub>NO<sub>13</sub>: C, 52.55; H, 5.19; N, 1.80%. Found: C, 52.48; H, 5.37; N, 1.67%.

**(3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranose trichloroacetimidate (306).** The hemiacetal **318** (539 mg, 0.68 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL), placed under argon and cooled to 0°C. Trichloroacetonitrile (0.6 mL, 6.8 mmol), then DBU (10  $\mu$  L, 70  $\mu$ mol) were added. The mixture was stirred at 0°C for 30 min. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was eluted from a column of silica gel with 7:3 cyclohexane-EtOAc and 0.2 % of Et<sub>3</sub>N to give **306** (498 mg, 78 %) as a colourless foam;  $[\alpha]_D -18^\circ$  (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  8.48 (s, 1H, NH), 7.40-7.15 (m, 10H, Ph), 6.75 (d, 1H,  $J_{2,NH} = 8.5$  Hz, NH<sub>D</sub>), 6.18 (d, 1H,  $J_{1,2} = 1.1$  Hz, H-1<sub>A</sub>), 5.15 (pt, 1H,  $J_{2,3} = J_{3,4} = 9.5$  Hz, H-3<sub>D</sub>), 5.07 (pt, 1H,  $J_{4,5} = 9.5$  Hz, H-4<sub>D</sub>), 4.82-4.50 (m, 4H, CH<sub>2</sub>Ph), 4.62 (d, 1H,  $J_{1,2} = 8.5$  Hz, H-1<sub>D</sub>), 4.20-4.03 (m, 2H,  $J_{5,6} = 4.5$ ,  $J_{6a,6b} = 12.3$  Hz, H-6a<sub>D</sub>, 6b<sub>D</sub>), 3.98 (m, 1H, H-2<sub>D</sub>), 3.85 (dq, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.2$  Hz, H-5<sub>A</sub>), 3.84 (dd, 1H,  $J_{2,3} = 3.3$  Hz, H-2<sub>A</sub>), 3.83 (dd, 1H,  $J_{3,4} = 9.5$  Hz, H-3<sub>A</sub>), 3.55 (m, 1H, H-5<sub>D</sub>), 3.45 (pt, 1H, H-4<sub>A</sub>), 1.98, 1.96, 1.93 (3s, 9H, OAc), 1.23 (d, 3H, H-6<sub>A</sub>); <sup>13</sup>C NMR:  $\delta$  171.1, 170.0, 169.6, 162.1 (C=O), 138.4-127.2 (Ph), 101.7 (C-1<sub>D</sub>), 97.2 (C-1<sub>A</sub>), 92.6 (CCl<sub>3</sub>), 80.5 (C-4<sub>A</sub>), 79.1 (C-3<sub>A</sub>), 76.2 (C-2<sub>A</sub>), 76.2, 74.1 (2C, CH<sub>2</sub>Ph), 74.4 (C-3<sub>D</sub>), 74.1 (C-5<sub>D</sub>), 71.3 (C-5<sub>A</sub>), 68.6 (C-4<sub>D</sub>), 62.3 (C-6<sub>D</sub>), 56.3 (C-2<sub>D</sub>), 21.1, 21.0, 20.9 (3C, OAc), 18.2 (C-6<sub>A</sub>). Anal. Calcd for C<sub>36</sub>H<sub>40</sub>Cl<sub>6</sub>N<sub>2</sub>O<sub>13</sub>: C, 46.93; H, 4.38; N, 3.04%. Found: C, 46.93; H, 4.52; N, 2.85%.

**Allyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranoside (319).** A mixture of the protected disaccharide **317** (3.0 g, 3.61 mmol) in MeOH (50 mL) was cooled to 0°C and treated by NH<sub>3</sub> gaz overnight. The solution was concentrated and the residue (2.02 g) was dissolved again in MeOH (50 mL) and treated by Ac<sub>2</sub>O (3.98 mL, 36.1  $\mu$ mol). The solution was

stirred for 2 h and then concentrated. The residue was eluted from a column of silica gel with 95:5 DCM-EtOAc to give the intermediate triol which was dissolved in Pyridine (5 mL), cold to 0°C and treated by Ac<sub>2</sub>O (2.4 mL). The mixture was stirred overnight and concentrated. The residue was eluted from a column of silica gel with 3:2 cyclohexane-EtOAc to give **319** (2.3 g, 90 %) was obtained as a colourless foam.  $[\alpha]_D -12^\circ$  (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  7.32-7.18 (m, 10H, Ph), 5.80-5.70 (m, 1H, All), 5.40 (d, 1H,  $J_{2,NH} = 8.1$  Hz, NH), 5.20-5.10 (m, 2H, All), 4.96 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.5$  Hz, H-4<sub>D</sub>), 4.90 (pt, 1H,  $J_{2,3} = 9.5$  Hz, H-3<sub>D</sub>), 4.80 (d, 1H,  $J_{1,2} = 1.2$  Hz, H-1<sub>A</sub>), 4.76-4.52 (m, 4H, CH<sub>2</sub>Ph), 4.46 (d, 1H,  $J_{1,2} = 8.5$  Hz, H-1<sub>D</sub>), 4.10-4.02 (m, 2H,  $J_{5,6} = 4.7$ ,  $J_{6a,6b} = 11.2$  Hz, H-6a<sub>D</sub>, 6b<sub>D</sub>), 3.92 (m, 1H, H-2<sub>D</sub>), 3.87 (m, 2H, All), 3.86 (dd, 1H,  $J_{2,3} = 3.5$  Hz, H-2<sub>A</sub>), 3.82 (dd, 1H,  $J_{3,4} = 9.5$  Hz, H-3<sub>A</sub>), 3.62 (dq, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.2$  Hz, H-5<sub>A</sub>), 3.52 (m, 1H, H-5<sub>D</sub>), 3.30 (pt, 1H, H-4<sub>A</sub>), 1.98, 1.94, 1.92 (3 s, 9H, OAc), 1.26 (d, 3H, H-6<sub>A</sub>); <sup>13</sup>C NMR:  $\delta$  171.1, 171.0, 170.3, 169.6 (C=O), 138-117 (Ph, All), 103.4 (C-1<sub>D</sub>), 98.5 (C-1<sub>A</sub>), 81.3 (C-4<sub>A</sub>), 80.4 (C-3<sub>A</sub>), 78.5 (C-2<sub>A</sub>), 75.9, 73.9 (2C, CH<sub>2</sub>Ph), 73.6 (C-3<sub>D</sub>), 72.4 (C-5<sub>D</sub>), 68.7 (C-4<sub>D</sub>), 68.2 (C-5<sub>A</sub>), 68.1 (All), 62.5 (C-6<sub>D</sub>), 54.5 (C-2<sub>D</sub>), 23.4 (NHAc), 21.2, 21.1, 21.0 (3C, OAc), 18.1 (C-6<sub>A</sub>). FAB-MS for C<sub>37</sub>H<sub>47</sub>NO<sub>13</sub> (M, 713.3)  $m/z$  736.2 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>37</sub>H<sub>47</sub>NO<sub>13</sub>: C, 62.26; H, 6.64; N, 1.96. Found: C, 62.12; H, 6.79; N, 1.87.

**(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranose (320).**

1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (10 mg, 12  $\mu$ mol) was dissolved THF (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **319** (830 mg, 1.16 mmol) in THF (40 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (90 mL), and water (10 mL) was added. Mercuric chloride (475 mg, 1.75 mmol) and mercuric oxide (504 mg, 2.32 mmol) were added to the mixture, protected from light. The mixture was stirred for 2 h at rt, then concentrated. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (cyclohexane-EtOAc 3:7) to give **320** (541 mg, 69 %) as a white foam;  $[\alpha]_D +16^\circ$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR:  $\delta$  7.35-7.05 (m, 10H, Ph), 5.50 (d, 1H,  $J_{2,NH} = 8.2$  Hz, NH<sub>D</sub>), 5.22 (d, 1H,  $J_{1,2} = 1.1$  Hz, H-1<sub>A</sub>), 5.06 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.5$  Hz, H-4<sub>D</sub>), 5.00 (pt, 1H,  $J_{2,3} = 9.5$  Hz, H-3<sub>D</sub>), 4.85-4.60 (m, 4H, CH<sub>2</sub>Ph), 4.56 (d, 1H,  $J_{1,2} = 7.0$  Hz, H-1<sub>D</sub>), 4.22-4.13 (m, 2H,  $J_{5,6} = 4.5$ ,  $J_{6a,6b} = 12.3$  Hz, H-6a<sub>D</sub>, 6b<sub>D</sub>), 4.03 (m, 1H, H-2<sub>D</sub>), 4.00 (dq, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.2$  Hz, H-5<sub>A</sub>), 3.96 (dd, 1H,  $J_{2,3} = 3.3$  Hz, H-2<sub>A</sub>), 3.90 (dd, 1H,  $J_{3,4} = 9.5$  Hz, H-3<sub>A</sub>), 3.60 (m, 1H, H-5<sub>D</sub>), 3.48 (d, 1H,  $J_{1,OH} = 3.5$  Hz, OH), 3.40 (pt, 1H, H-4<sub>A</sub>), 2.08, 2.03, 2.01 (3s, 9H, OAc), 1.65 (s, 3H, NHAc), 1.30 (d, 3H, H-6<sub>A</sub>); <sup>13</sup>C NMR:  $\delta$  171.2, 171.0, 170.4, 169.6 (C=O), 138.2-128.0 (Ph), 103.3 (C-1<sub>D</sub>), 94.1

(C-1<sub>A</sub>), 81.4 (C-4<sub>A</sub>), 79.9 (C-2<sub>A</sub>), 78.7 (C-3<sub>A</sub>), 75.8, 73.9 (2C, CH<sub>2</sub>Ph), 73.6 (C-3<sub>D</sub>), 72.4 (C-5<sub>D</sub>), 68.7 (C-4<sub>D</sub>), 68.2 (C-5<sub>A</sub>), 62.4 (C-6<sub>D</sub>), 54.5 (C-2<sub>D</sub>), 23.3 (NHAc), 21.1, 21.0, 21.1 (3C, OAc), 18.3 (C-6<sub>A</sub>). FAB-MS for C<sub>34</sub>H<sub>43</sub>NO<sub>13</sub> (M, 673.2), *m/z* 696.3 [M+Na]<sup>+</sup>. Anal Calcd for C<sub>34</sub>H<sub>43</sub>NO<sub>13</sub>: C, 60.61; H, 6.43; N, 2.08. Found: C, 60.46; H, 6.61; N, 1.95.

- 5                   **(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranose trichloroacetimidate (307).** The hemiacetal **320** (541 mg, 0.80 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL), placed under argon and cooled to 0°C. Trichloroacetoneitrile (0.810 mL, 8 mmol), then DBU (10 μL, 80 μmol) were added. The mixture was stirred at 0°C for 1 h. The mixture was concentrated and toluene was co-
- 10 evaporated from the residue. The residue was eluted from a column of silica gel with 1:1 cyclohexane-EtOAc and 0.2 % of Et<sub>3</sub>N to give **307** (560 mg, 86 %) as a colourless foam; [ $\alpha$ ]<sub>D</sub> +2° (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  8.56 (s, 1H, NH), 7.50-7.20 (m, 10H, Ph), 6.29 (d, 1H, *J*<sub>1,2</sub> = 1.3 Hz, H-1<sub>A</sub>), 5.50 (d, 1H, *J*<sub>2,NH</sub> = 8.3 Hz, NH<sub>D</sub>), 5.17 (pt, 1H, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = 9.5 Hz, H-3<sub>D</sub>), 5.09 (dd, 1H, *J*<sub>4,5</sub> = 9.5 Hz, H-4<sub>D</sub>), 4.85-4.60 (m, 4H, CH<sub>2</sub>Ph), 4.68 (d, 1H, *J*<sub>1,2</sub> = 8.0
- 15 Hz, H-1<sub>D</sub>), 4.22-4.10 (m, 2H, *J*<sub>5,6</sub> = 5.0, *J*<sub>6a,6b</sub> = 12.2 Hz, H-6a<sub>D</sub>, 6b<sub>D</sub>), 4.00 (m, 1H, H-2<sub>D</sub>), 3.99 (dd, 1H, *J*<sub>2,3</sub> = 3.5 Hz, H-2<sub>A</sub>), 3.90 (dq, 1H, *J*<sub>4,5</sub> = 9.6, *J*<sub>5,6</sub> = 6.2 Hz, H-5<sub>A</sub>), 3.89 (dd, 1H, *J*<sub>3,4</sub> = 9.5 Hz, H-3<sub>A</sub>), 3.62 (m, 1H, H-5<sub>D</sub>), 3.50 (dd, 1H, H-4<sub>A</sub>), 2.02, 2.00, 1.98 (3s, 9H, OAc), 1.65 (s, 3H, NHAc), 1.32 (d, 3H, H-6<sub>A</sub>); <sup>13</sup>C NMR:  $\delta$  171.2, 171.0, 170.4, 169.6 (C=O), 160.5 (C=NH), 138.2-128.0 (Ph), 103.3 (C-1<sub>D</sub>), 97.3 (C-1<sub>A</sub>), 91.4 (CCl<sub>3</sub>), 80.3 (C-
- 20 4<sub>A</sub>), 79.9 (C-3<sub>A</sub>), 77.5 (C-2<sub>A</sub>), 76.0, 73.8 (2C, CH<sub>2</sub>Ph), 73.1 (C-3<sub>D</sub>), 72.2 (C-5<sub>D</sub>), 71.1 (C-5<sub>A</sub>), 68.8 (C-4<sub>D</sub>), 62.5 (C-6<sub>D</sub>), 54.8 (C-2<sub>D</sub>), 23.3 (NHAc), 21.4, 21.1, 21.0 (3C, OAc), 18.4 (C-6<sub>A</sub>). Anal. Calcd for C<sub>36</sub>H<sub>43</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>13</sub>: C, 52.85; H, 5.30; N, 3.42. Found: C, 52.85; H, 5.22; N, 3.47.

- Allyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (322).** The acceptor **314** (1.78 g, 4.65 mmol) and the trichloroacetimidate donor **321** (2.96 g, 5.58 mmol) were dissolved in anhydrous ether (100 mL). The mixture was placed under argon and cooled to -55°C. TMSOTf (335 μL, 1.86 mmol) was added dropwise. The mixture was stirred at -55°C to -20°C over 3 h. Triethylamine (0.75 mL) was added, and the mixture was allowed to warm to rt. The
- 30 mixture was concentrated. The residue was purified by column chromatography (cyclohexane:EtOAc, 7:3) to give **322** as a colourless syrup (3.21 g, 92 %); [ $\alpha$ ]<sub>D</sub> -16° (*c* 0.55, CHCl<sub>3</sub> lit. Zhang, J.; Mao, J. M.; Chen, H. M.; Cai, M. S. *Tetrahedron: Asymmetry* **1994**, 5, 2283-2290) [ $\alpha$ ]<sub>D</sub> -19.3° (*c* 1.2, CHCl<sub>3</sub>); <sup>1</sup>H NMR:  $\delta$  7.42-7.30 (m, 20H, Ph), 5.92-5.82 (m, 1H, All), 5.62 (dd, 1H, *J*<sub>1,2</sub> = 1.6, *J*<sub>2,3</sub> = 3.2 Hz, H-2<sub>A</sub>), 5.32-5.20 (m, 2H, All), 5.07 (d, 1H, H-1<sub>A</sub>), 4.82 (d, 1H, *J*<sub>1,2</sub> = 1.0 Hz, H-1<sub>B</sub>), 4.95-4.60 (m, 8H, CH<sub>2</sub>Ph), 4.20-
- 35 4.15 (m, 1H, All), 4.09 (d, 1H, *J*<sub>2,3</sub> = 3.0 Hz, H-2<sub>B</sub>), 4.05 (dd, 1H, *J*<sub>3,4</sub> = 9.4 Hz, H-3<sub>A</sub>), 4.05-3.95 (m, 1H, All), 3.96 (dd, 1H, *J*<sub>3,4</sub> = 9.5 Hz, H-3<sub>B</sub>), 3.89 (dq, 1H, *J*<sub>4,5</sub> = 9.5, *J*<sub>5,6</sub> = 6.3 Hz, H-5<sub>A</sub>), 3.76 (dq, 1H, *J*<sub>4,5</sub> = 9.5, *J*<sub>5,6</sub> = 6.2 Hz, H-5<sub>B</sub>), 3.52 (m, 1H, H-4<sub>B</sub>), 3.50 (m,

1H, H-4<sub>A</sub>), 2.18 (s, 3H, OAc), 1.39 (d, 3H, H-6<sub>A</sub>), 1.36 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR: δ 170.8 (C=O), 138.4-117.1 (Ph, All), 99.5 (C-1<sub>A</sub>), 98.4 (C-1<sub>B</sub>), 80.5 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 80.0 (C-3<sub>B</sub>), 78.1 (C-3<sub>A</sub>), 75.8, 75.7 (2C, CH<sub>2</sub>Ph), 74.9 (C-2<sub>B</sub>), 72.5, 72.2 (2C, CH<sub>2</sub>Ph), 69.3 (C-2<sub>A</sub>), 68.6 (C-5<sub>A</sub>), 68.4 (C-5<sub>B</sub>), 68.0 (All), 21.5 (OAc), 18.4, 18.2 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). CI-MS for C<sub>45</sub>H<sub>52</sub>O<sub>10</sub> (M, 752) *m/z* 770 [M + NH<sub>4</sub>]<sup>+</sup>. Anal. Calcd. for C<sub>45</sub>H<sub>52</sub>O<sub>10</sub>: C, 71.79; H, 6.96. Found: C, 70.95; H, 7.01.

**Allyl (3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (323).** A 1M solution of sodium methoxide in methanol (1.1 mL) was added to a solution of **322** (3.10 g, 4.13 mmol) in methanol. The mixture was stirred at rt for 3 h. The mixture was neutralised with Amberlite IR-120 (H<sup>+</sup>) resin, filtered and concentrated to give **323** (2.72 g, 93%) as a colourless syrup which crystallised on standing; mp 98-99°C; lit. (Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Org. Chem.* **1989**, *54*, 2650-2656) mp 100°C (hexane); [α]<sub>D</sub> -30° (c 0.5, CHCl<sub>3</sub>), lit. (Pinto, B. M.; Reimer, K. B.; Morissette, D. G.; Bundle, D. R. *J. Org. Chem.* **1989**, *54*, 2650-2656) [α]<sub>D</sub> -32.5° (c, 0.4, CHCl<sub>3</sub>); <sup>1</sup>H NMR: δ 7.42-7.30 (m, 20H, Ph), 5.90-5.80 (m, 1H, All), 5.32-5.20 (m, 2H, All), 5.13 (d, 1H, *J*<sub>1,2</sub> = 1.4 Hz, H-1<sub>A</sub>), 4.82 (d, 1H, *J*<sub>1,2</sub> = 1.6 Hz, H-1<sub>B</sub>), 4.95-4.60 (m, 8H, CH<sub>2</sub>Ph), 4.20-4.12 (m, 1H, All), 4.19 (m, 1H, *J*<sub>2,3</sub> = 3.2, *J*<sub>2,OH</sub> = 1.8 Hz, H-2<sub>A</sub>), 4.09 (d, 1H, *J*<sub>2,3</sub> = 3.2 Hz, H-2<sub>B</sub>), 4.00-3.95 (m, 1H, All), 3.95 (dd, 1H, *J*<sub>3,4</sub> = 9.4 Hz, H-3<sub>A</sub>), 3.93 (dd, 1H, *J*<sub>3,4</sub> = 9.4 Hz, H-3<sub>B</sub>), 3.87 (dq, 1H, *J*<sub>4,5</sub> = 9.4, *J*<sub>5,6</sub> = 6.2 Hz, H-5<sub>A</sub>), 3.74 (dq, 1H, *J*<sub>4,5</sub> = 9.4, *J*<sub>5,6</sub> = 6.2 Hz, H-5<sub>B</sub>), 3.53 (pt, 1H, H-4<sub>A</sub>), 3.46 (pt, 1H, H-4<sub>B</sub>), 2.52 (d, 1H, OH), 1.35 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR: δ 138.4-117.1 (Ph, All), 101.2 (C-1<sub>A</sub>), 98.4 (C-1<sub>B</sub>), 80.8, 80.4 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 80.3 (C-3<sub>B</sub>), 80.0 (C-3<sub>A</sub>), 75.8, 75.7 (2C, CH<sub>2</sub>Ph), 75.0 (C-2<sub>B</sub>), 72.7, 72.6 (2C, CH<sub>2</sub>Ph), 69.1 (C-2<sub>A</sub>), 68.4 (C-5<sub>B</sub>), 68.3 (C-5<sub>A</sub>), 68.1 (All), 18.4, 18.3 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). CI-MS for C<sub>43</sub>H<sub>50</sub>O<sub>9</sub> (M, 710) *m/z* 728 [M + NH<sub>4</sub>]<sup>+</sup>.

**3,4,6-Tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl Trichloroacetimidate (324)** (Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36*, 5343-5346). Trichloroacetonitrile (2.5 mL) and anhydrous potassium carbonate were added to a suspension of 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-α/β-D-glucopyranose (7.88 g, 13.75 mmol) in 1,2-DCE (120 mL). The mixture was stirred at rt overnight. TLC (cyclohexane:EtOAc, 3:2) showed that no starting material remained. The mixture was filtered through a pad of Celite, and the filtrate was concentrated to give the target **324** as a slightly brownish solid (9.08 g, 92%).

**Allyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (325).** 1,2-DCE (35 mL) was added to the trichloroacetimidate donor **316** (2.49 g, 4.20 mmol), the acceptor **323** (2.48 g, 3.50 mmol) and 4Å powdered molecular sieves (4 g). The mixture was stirred for 1.5 h at rt under Argon. The mixture was cooled to -20°C and TMSOTf (230 μL, 1.26 mmol) was added.

The temperature was allowed to reach 0°C over 1 h, and the mixture was stirred for an additional 2 h at this temperature. Triethylamine (0.5 mL) was added and the mixture was allowed to warm to rt. The mixture was diluted with DCM and filtered. The filtrate was concentrated. The residue was purified by column chromatography with 3:1 cyclohexane-EtOAc to give **325** (3.83 g, 96 %) as a colourless amorphous solid:  $[\alpha]_D -6^\circ$  (*c* 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR: δ 7.52-7.28 (m, 20H, Ph), 6.83 (d, 1H, *J*<sub>2,NH</sub> = 8.4 Hz, NH), 5.85 (m, 1H, All), 5.26-5.09 (m, 4H, H-3<sub>D</sub>, 4<sub>D</sub>, All), 4.98 (d, 1H, *J*<sub>1,2</sub> = 1.4 Hz, H-1<sub>A</sub>), 4.98-4.58 (m, 10H, H-1<sub>B</sub>, 1<sub>D</sub>, CH<sub>2</sub>Ph), 4.08 (m, 4H, H-2<sub>A</sub>, 2<sub>D</sub>, 6a<sub>D</sub>, All), 3.91 (m, 5H, H-2<sub>B</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 6b<sub>D</sub>, All), 3.79 (m, 2H, H-5<sub>A</sub>, 5<sub>B</sub>), 3.45 (m, 3H, H-4<sub>A</sub>, 4<sub>B</sub>, 5<sub>D</sub>), 2.04, 2.02, 1.97 (3s, 9H, OAc), 1.30 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR: δ 170.6, 170.3, 169.1, 161.6 (C=O), 138.4-117.1 (Ph, All), 101.3 (C-1<sub>D</sub>), 100.9 (C-1<sub>A</sub>), 97.6 (C-1<sub>B</sub>), 92.0 (CCl<sub>3</sub>), 80.9, 80.4 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 79.1, 79.0 (2C, C-3<sub>A</sub>, 3<sub>B</sub>), 77.3 (C-2<sub>A</sub>), 76.5 (C-2<sub>B</sub>), 75.4, 75.2, 73.6 (3C, CH<sub>2</sub>Ph), 72.2 (C-3<sub>D</sub>), 71.9 (C-5<sub>D</sub>), 71.6 (CH<sub>2</sub>Ph), 68.2 (C-5<sub>B</sub>\*), 67.8 (C-4<sub>D</sub>), 67.5 (C-5<sub>A</sub>\*), 67.5 (CH<sub>2</sub>O), 61.3 (C-6<sub>D</sub>), 55.7 (C-2<sub>D</sub>), 20.5, 20.4 (3C, OAc), 17.9, 17.7 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>57</sub>H<sub>66</sub>Cl<sub>3</sub>NO<sub>17</sub> (M, 1141.3) *m/z* 1164.3 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>57</sub>H<sub>66</sub>Cl<sub>3</sub>NO<sub>17</sub>: C, 59.87; H, 5.82; N, 1.22%. Found: C, 59.87; H, 5.92; N, 1.16%.

**Allyl (3,4,6-Tri-O-acetyl-2-deoxy-2-tetrachlorophthalimido-β-D-glucopyranosyl)-(1→2)-(3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (328).** Anhydrous Et<sub>2</sub>O (30 mL) and DCM (15 mL) were added to the trichloroacetimidate donor **324** (3.34 g, 4.66 mmol), the acceptor **323** (2.20 g, 3.10 mmol). The mixture was cooled to 0°C and TMSOTf (85 μL, 0.466 mmol) was added dropwise. The mixture was stirred at 0°C for 1 h, then at rt for 3 h. Triethylamine (1 mL) was added and the mixture was stirred for 10 min, then concentrated. The mixture was taken up in Et<sub>2</sub>O and the resulting precipitate was filtered off. The filtrate was concentrated. The residue was purified by column chromatography with 7:3 cyclohexane-EtOAc to give **328** (2.57 g, 65 %) as a colourless amorphous solid:  $[\alpha]_D +22^\circ$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz): δ 7.42-7.16 (m, 20H, Ph), 5.91 (dd, 1H, H-3<sub>D</sub>), 5.81 (m, 1H, All), 5.24-5.10 (m, 4H, H-1<sub>D</sub>, 4<sub>D</sub>, All), 4.93 (s, 1H, H-1<sub>A</sub>), 4.81-4.53 (m, 5H, H-1<sub>B</sub>, CH<sub>2</sub>Ph), 4.45-4.23 (m, 5H, H-2<sub>D</sub>, CH<sub>2</sub>Ph), 4.05 (m, 2H, H-6a<sub>D</sub>, All), 3.91-3.58 (m, 8H, H-2<sub>A</sub>, 2<sub>B</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 6b<sub>D</sub>, All), 3.38 (m, 1H, H-5<sub>D</sub>), 3.21-3.13 (m, 2H, H-4<sub>A</sub>, 4<sub>B</sub>), 2.05, 2.02, 2.00 (3s, 9H, OAc), 1.24 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR (75 MHz): δ 170.5, 170.4, 169.3 (C=O), 138.4-117.1 (Ph, All), 101.1 (C-1<sub>A</sub>), 99.9 (C-1<sub>D</sub>), 97.7 (C-1<sub>B</sub>), 80.6 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 79.7, 78.9 (2C, C-3<sub>A</sub>, 3<sub>B</sub>), 78.2 (C-2<sub>A</sub>), 76.3 (C-2<sub>B</sub>), 75.2, 75.1, 72.6, 71.3 (4C, CH<sub>2</sub>Ph), 71.2 (C-5<sub>D</sub>), 70.1 (C-3<sub>D</sub>), 68.4 (C-5<sub>B</sub>\*), 68.4 (C-4<sub>D</sub>), 67.6 (C-5<sub>A</sub>\*), 67.6 (All), 61.3 (C-6<sub>D</sub>), 55.4 (C-2<sub>D</sub>), 20.6, 20.5 (3C, OAc), 18.0, 17.6 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>63</sub>H<sub>65</sub>Cl<sub>4</sub>NO<sub>18</sub> (M, 1263.3) *m/z* 1288.4, 1286.4 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>63</sub>H<sub>65</sub>Cl<sub>4</sub>NO<sub>18</sub>: C, 59.77; H, 5.17; N, 1.11%. Found: C, 60.19; H, 5.53; N, 1.18%.

**Allyl (2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranoside (326)**

The trisaccharide 325 (1.71 g, 1.50 mmol) was dissolved in MeOH (20 mL). A 1 M solution of sodium methoxide in methanol (9 mL) and triethylamine (5 mL) were added and the mixture was stirred at rt for 18 h. The mixture was cooled to 0°C and acetic anhydride was added dropwise until the pH reached 6. A further portion of acetic anhydride (0.4 mL) was added, and the mixture was stirred at rt for 30 min. The mixture was concentrated, and toluene was co-evaporated from the residue. The residue was purified by column chromatography with 95:5 DCM-MeOH to give 326 (623 mg, 45 %) as a colourless amorphous solid:  $[\alpha]_D -16^\circ$  (*c* 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz):  $\delta$  7.48-7.24 (m, 20H, Ph), 6.79 (d, 1H, NH), 5.73 (m, 1H, All), 5.12 (m, 3H, H-1<sub>A</sub>, All), 4.86-4.52 (m, 9H, H-1<sub>B</sub>, CH<sub>2</sub>Ph), 4.34 (d, 1H, H-1<sub>D</sub>), 4.08-3.79 (m, 6H, H-2<sub>A</sub>, 2<sub>B</sub>, 3<sub>A</sub>, 3<sub>B</sub>, All), 3.74-3.53 (m, 3H, H-5<sub>A</sub>, 5<sub>B</sub>, 6a<sub>D</sub>), 3.45-3.24 (m, 6H, H-2<sub>D</sub>, 3<sub>D</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>D</sub>, 6b<sub>D</sub>), 3.20 (m, 1H, H-5<sub>D</sub>), 1.46 (s, 3H, NHAc), 1.24 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR (75 MHz):  $\delta$  173.6 (C=O), 137.4-117.3 (Ph, All), 103.2 (C-1<sub>D</sub>), 100.3 (C-1<sub>A</sub>), 97.9 (C-1<sub>B</sub>), 81.3, 80.4 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 79.9 (2C, C-3<sub>A</sub>, 3<sub>B</sub>), 79.9 (C-2<sub>B</sub>\*), 78.9 (C-3<sub>D</sub>), 75.7 (C-5<sub>D</sub>), 75.6, 75.3, 74.5 (3C, CH<sub>2</sub>Ph), 73.6 (C-2<sub>A</sub>\*), 72.5 (CH<sub>2</sub>Ph), 71.9 (C-4<sub>D</sub>), 68.2, 68.0 (2C, C-5<sub>A</sub>, 5<sub>B</sub>), 67.7 (CH<sub>2</sub>O), 62.5 (C-6<sub>D</sub>), 58.8 (C-2<sub>D</sub>), 22.3 (NHAc), 18.0, 17.8 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>51</sub>H<sub>63</sub>NO<sub>14</sub> (M, 913.4) *m/z* 936.6 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>51</sub>H<sub>63</sub>NO<sub>14</sub>·H<sub>2</sub>O: C, 65.72; H, 7.03; N, 1.50%. Found: C, 65.34; H, 7.03; N, 1.55%.

**Allyl (2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranoside (327).** (a) Pyridine (5 mL) was added to 326 (502 mg, 0.55 mmol) and the mixture was cooled to 0°C. Acetic anhydride (3 mL) was added. The mixture was stirred at rt for 18 h. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was taken up in DCM and washed successively with 5% aq HCl and saturated aq NaHCO<sub>3</sub>. The organic phase was dried and concentrated to give 327 (538 mg, 94 %) as a colourless foam.

(b) THF (3 mL) and ethanol (3.3 mL) were added to 328 (384 mg, 0.30 mmol). Ethylenediamine (90  $\mu$ L, 1.36 mmol) was added and the mixture was heated at 55°C for 4 h. The mixture was allowed to cool to rt. Acetic anhydride (1.0 mL) was added, and the mixture was stirred at rt for 1.5 h. The mixture was concentrated. The residue was taken up in pyridine (5 mL) and the mixture was cooled to 0°C. Acetic anhydride (2.5 mL) was added. The mixture was stirred at rt for 18 h. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was taken up in DCM, which caused the formation of a white precipitate. The mixture was filtered through a plug of silica gel, eluting with 7:3 cyclohexane-acetone. The filtrate was concentrated to give 327 (215 mg, 68 %) as a colourless foam:  $[\alpha]_D -7^\circ$  (*c* 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz):  $\delta$



7.48-7.24 (m, 20H, Ph), 5.84 (m, 1H, All), 5.53 (d, 1H, NH), 5.19 (m, 2H, All), 5.03 (dd, 1H, H-4<sub>D</sub>), 4.98 (m, 2H, H-1<sub>A</sub>, 3<sub>D</sub>), 4.95-4.54 (m, 10H, H-1<sub>B</sub>, 1<sub>D</sub>, CH<sub>2</sub>Ph), 4.07 (m, 4H, H-2<sub>A</sub>, 2<sub>D</sub>, 6a<sub>D</sub>, All), 3.88 (m, 5H, H-2<sub>B</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 6b<sub>D</sub>, All), 3.79, 3.68 (2m, 2H, H-5<sub>A</sub>, 5<sub>B</sub>), 3.42 (m, 3H, H-4<sub>A</sub>, 4<sub>B</sub>, 5<sub>D</sub>), 2.02, 2.01, 1.97, 1.64 (4s, 12H, OAc, NHAc), 1.30 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR (75 MHz): δ 170.7, 170.4, 169.9, 169.1 (C=O), 138.5-117.1 (Ph, All), 102.9 (C-1<sub>D</sub>), 101.2 (C-1<sub>A</sub>), 97.7 (C-1<sub>B</sub>), 81.0, 80.5 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 79.5, 79.1 (2C, C-3<sub>A</sub>, 3<sub>B</sub>), 78.2 (C-2<sub>A</sub>), 76.1 (C-2<sub>B</sub>), 75.5, 75.2, 73.6 (CH<sub>2</sub>Ph), 73.3 (C-3<sub>D</sub>), 71.9 (C-5<sub>D</sub>), 71.7 (CH<sub>2</sub>Ph), 68.3 (C-5<sub>A</sub>\*), 68.0 (C-4<sub>D</sub>), 67.6 (C-5<sub>B</sub>\*), 67.6 (CH<sub>2</sub>O), 61.6 (C-6<sub>D</sub>), 54.1 (C-2<sub>D</sub>), 22.9 (NHAc), 20.7, 20.6 (3C, OAc), 18.0, 17.7 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>57</sub>H<sub>69</sub>NO<sub>17</sub> (M, 1039.5) *m/z* 1062.4 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>57</sub>H<sub>69</sub>NO<sub>17</sub>: C, 65.82; H, 6.69; N, 1.35%. Found: C, 65.29; H, 6.82; N, 1.29%.

(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α/β-L-rhamnopyranose (329). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (30 mg, 35 μmol) was dissolved THF (5 mL), and the resulting red solution was processed as described for the preparation of 318. A solution of 327 (805 mg, 0.775 mmol) in THF (10 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated. The residue was taken up in acetone (15 mL) and water (1.5 mL). Mercuric chloride (315 mg, 1.16 mmol) and mercuric oxide (335 mg, 1.55 mmol) were added. The mixture, protected from light, was stirred for 1 h at rt, then concentrated. The residue was taken up in DCM and washed three times with satd aqueous KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography with 2:3 EtOAc-cyclohexane to give 329 (645 mg, 83 %) as a white foam. The <sup>1</sup>H NMR spectra showed the α:β ratio to be 3.3:1; [α]<sub>D</sub> +3° (c 0.5, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz) α-anomer: δ 7.47-7.30 (m, 20H, Ph), 5.53 (d, 1H, NH), 5.17 (d, 1H, *J*<sub>1,2</sub> = 1.9 Hz, H-1<sub>B</sub>), 5.08 (m, 1H, H-4<sub>D</sub>), 5.03 (d, 1H, *J*<sub>1,2</sub> = 1.5 Hz, H-1<sub>A</sub>), 4.99 (m, 1H, H-3<sub>D</sub>), 4.92-4.62 (m, 8H, CH<sub>2</sub>Ph), 4.60 (d, 1H, *J*<sub>1,2</sub> = 8.4 Hz, H-1<sub>D</sub>), 4.18-4.01 (m, 3H, H-2<sub>A</sub>, 2<sub>D</sub>, 6a<sub>D</sub>), 3.97-3.90 (m, 5H, H-2<sub>B</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 5<sub>A</sub>\*, 6b<sub>D</sub>), 3.83 (m, 1H, H-5<sub>B</sub>\*), 3.45-3.37 (m, 3H, H-4<sub>A</sub>, 4<sub>B</sub>, 5<sub>D</sub>), 2.04, 2.03, 1.99, 1.68 (4s, 12H, OAc, NHAc), 1.32 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR (75 MHz) α-anomer: δ 170.7, 170.4, 169.9, 169.1 (C=O), 138.5-129.3 (Ph), 103.3 (C-1<sub>D</sub>), 101.6 (C-1<sub>A</sub>), 93.9 (C-1<sub>B</sub>), 81.5, 80.8 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 79.9, 78.9 (2C, C-3<sub>A</sub>, 3<sub>B</sub>), 78.6 (C-2<sub>A</sub>), 76.8 (C-2<sub>B</sub>), 76.0, 75.5, 74.0 (3C, CH<sub>2</sub>Ph), 73.7 (C-3<sub>D</sub>), 72.4 (C-5<sub>D</sub>), 72.2 (CH<sub>2</sub>Ph), 68.7 (C-5<sub>A</sub>\*), 68.5 (C-4<sub>D</sub>), 68.2 (C-5<sub>B</sub>\*), 62.0 (C-6<sub>D</sub>), 54.6 (C-2<sub>D</sub>), 23.4 (NHAc), 21.1, 21.0 (3C, OAc), 18.5, 18.1 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>54</sub>H<sub>65</sub>NO<sub>17</sub> (M, 999.4) *m/z* 1022.5 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>54</sub>H<sub>65</sub>NO<sub>17</sub>: C, 64.85; H, 6.55; N, 1.40%. Found: C, 64.55; H, 7.16; N, 1.15%.

(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α/β-L-

**rhamnopyranosyl Trichloroacetimidate (313).** The hemiacetal **329** (595 mg, 0.59 mmol) was dissolved in DCM (10 mL), placed under Argon and cooled to 0°C. Trichloroacetonitrile (0.6 mL, 6 mmol), then DBU (10 µL, 59 µmol) were added. The mixture was stirred at 0°C for 20 min, then at rt for 20 min. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was purified by flash chromatography with 1:1 cyclohexane-EtOAc and 0.2 % of Et<sub>3</sub>N to give **313** (634 mg, 94 %) as a colorless foam. The <sup>1</sup>H NMR spectra showed the α:β ratio to be 10:1: [α]<sub>D</sub> -20° (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz) α-anomer: δ 8.47 (s, 1H, C=NH), 7.38-7.20 (m, 20H, Ph), 6.10 (d, 1H, J<sub>1,2</sub> = 1.3 Hz, H-1<sub>B</sub>), 5.40 (d, 1H, NH), 5.01 (m, 1H, H-4<sub>D</sub>), 4.95 (d, 1H, J<sub>1,2</sub> = 1.2 Hz, H-1<sub>A</sub>), 4.89 (m, 1H, H-3<sub>D</sub>), 4.85-4.55 (m, 9H, H-1<sub>D</sub>, CH<sub>2</sub>Ph), 4.07 (dd, 1H, H-6a<sub>D</sub>), 4.03 (m, 1H, H-2<sub>A</sub>), 3.97 (m, 1H, H-2<sub>D</sub>), 3.91 (dd, 1H, H-6b<sub>D</sub>), 3.85-3.71 (m, 5H, H-2<sub>B</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 5<sub>A</sub>, 5<sub>B</sub>), 3.45-3.31 (m, 3H, H-4<sub>A</sub>, 4<sub>B</sub>, 5<sub>D</sub>), 1.99, 1.96, 1.91, 1.58 (4s, 12H, OAc, NHAc), 1.26 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR (75 MHz): δ 171.1, 170.9, 170.3, 169.6 (C=O), 160.6 (C=NH), 138.6-128.1 (Ph), 103.3 (C-1<sub>D</sub>), 101.6 (C-1<sub>A</sub>), 96.9 (C-1<sub>B</sub>), 91.3 (CCl<sub>3</sub>), 81.4, 80.2 (2C, C-4<sub>A</sub>, 4<sub>B</sub>), 79.9, 78.5 (2C, C-3<sub>A</sub>, 3<sub>B</sub>), 78.3 (C-2<sub>A</sub>), 75.9 (2C, CH<sub>2</sub>Ph), 75.0 (C-2<sub>B</sub>), 73.7 (CH<sub>2</sub>Ph), 73.7 (C-3<sub>D</sub>), 72.4 (CH<sub>2</sub>Ph), 72.4 (C-5<sub>D</sub>), 71.0, 69.0 (2C, C-5<sub>A</sub>, 5<sub>B</sub>), 68.5 (C-4<sub>D</sub>), 62.1 (C-6<sub>D</sub>), 54.6 (C-2<sub>D</sub>), 23.4 (NHAc), 21.1, 21.0 (3C, OAc), 18.5, 18.0 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). Anal. Calcd. for C<sub>56</sub>H<sub>65</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>17</sub>: C, 58.77; H, 5.72; N, 2.45%. Found: C, 58.78; H, 5.83; N, 2.45%.

**Allyl (2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl-(1→4)]-2-O-benzoyl-α-L-rhamnopyranoside (305).** Anhydrous Et<sub>2</sub>O (5 mL) was added to the donor **313** (500 mg, 0.44 mmol) and the acceptor **311** (Segat, F.; Mulard, L. A. *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) (242 mg, 0.29 mmol) and powdered 4Å molecular sieves. The mixture was placed under Argon and cooled to 0°C. Boron trifluoride etherate (415 µL, 3.27 mmol) was added. The mixture was stirred at 0°C for 1 h, then at rt for 18 h. The mixture was diluted with DCM and triethylamine (1 mL) was added. The mixture was filtered through a pad of Celite and the filtrate was concentrated. The residue was purified by column chromatography with 3:2 cyclohexane-EtOAc to give, in order, the acceptor **311** (132 mg, 54 %), **305** (231 mg, 44 %) and the hemiacetal **329** (129 mg, 29 %). The desired pentasaccharide **305** was obtained as a colourless foam: [α]<sub>D</sub> +10° (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR: δ 8.02-7.09 (m, 45H, Ph), 5.92 (m, 1H, All), 5.65 (d, 1H, NH), 5.37 (m, 1H, H-2<sub>C</sub>), 5.19 (m, 2H, All), 5.13 (bs, 1H, H-1<sub>A</sub>), 4.96-4.35 (m, 15H, H-1<sub>B</sub>, 1<sub>C</sub>, 1<sub>D</sub>, 1<sub>E</sub>, 2<sub>B</sub>, 3<sub>D</sub>, 4<sub>D</sub>, CH<sub>2</sub>Ph), 4.17 (m, 2H, H-2<sub>A</sub>, All), 4.04-3.87 (m, 8H, H-2<sub>D</sub>, 3<sub>A</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 5<sub>A</sub>, 5<sub>E</sub>, 6a<sub>D</sub>, All), 3.81-3.63 (m, 7H, H-3<sub>B</sub>, 4<sub>C</sub>, 4<sub>E</sub>, 5<sub>C</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 6b<sub>D</sub>), 3.59 (m, 1H, H-5<sub>B</sub>), 3.43 (m, 3H, H-2<sub>E</sub>, 4<sub>A</sub>, 5<sub>D</sub>), 3.28 (pt, 1H, H-4<sub>B</sub>), 2.01, 1.99, 1.71, 1.66 (4s, 12H, OAc, NHAc), 1.34 (m, 6H, H-6<sub>A</sub>, 6<sub>C</sub>), 1.00 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR: δ 170.5, 170.0, 169.3, 165.8, 163.5 (C=O), 138.7-

117.6 (Ph, All), 102.7 (C-1<sub>D</sub>), 100.8 (2C, C-1<sub>A</sub>, 1<sub>B</sub>), 98.1 (C-1<sub>E</sub>), 95.9 (C-1<sub>C</sub>), 81.8 (C-3<sub>E</sub>), 81.2 (2C, C-2<sub>E</sub>, 4<sub>A</sub>), 80.0 (C-4<sub>B</sub>), 79.7 (2C, C-3<sub>A</sub>, 3<sub>C</sub>), 78.2 (C-3<sub>B</sub>), 77.7 (C-2<sub>A</sub>), 77.3 (2C, C-4<sub>C</sub>, 4<sub>E</sub>), 75.6, 75.4, 74.9 (CH<sub>2</sub>Ph), 74.3 (C-2<sub>B</sub>), 73.8 (CH<sub>2</sub>Ph), 73.7 (C-3<sub>D</sub>), 72.8 (CH<sub>2</sub>Ph), 72.3 (C-2<sub>C</sub>), 72.1 (C-5<sub>D</sub>), 71.5 (C-5<sub>E</sub>), 70.2 (CH<sub>2</sub>Ph), 68.5 (C-5<sub>B</sub>), 68.4 (C-5<sub>A</sub>, CH<sub>2</sub>O), 68.2 (C-4<sub>D</sub>), 67.9 (C-6<sub>E</sub>), 67.4 (C-5<sub>C</sub>), 61.8 (C-6<sub>D</sub>), 54.3 (C-2<sub>D</sub>), 23.1 (NHAc), 20.7, 20.6, 20.4 (3C, OAc), 18.6 (C-6<sub>A</sub>), 18.0 (C-6<sub>C</sub>), 17.8 (C-6<sub>B</sub>). FAB-MS for C<sub>104</sub>H<sub>117</sub>NO<sub>27</sub> (M, 1812.1) *m/z* 1836.2, 1835.2 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>104</sub>H<sub>117</sub>NO<sub>27</sub>: C, 68.90; H, 6.50; N, 0.77%. Found: C, 68.64; H, 6.66; N, 1.05%.

**Allyl (3,4-Di-*O*-benzyl-2-*O*-chloroacetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranoside (332).** To a mixture of 323 (3.8 g, 5.35 mmol) in pyridine (40 mL) was added chloroacetic anhydride (1.83 g, 10.7 mmol) at 0°C. The solution was stirred overnight at 0°C. MeOH (10 mL) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 95:5 cyclohexane-acetone to give 332 (2.4 g, 57 %) as a colorless syrup: [ $\alpha$ ]<sub>D</sub> -15° (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR:  $\delta$  7.30-7.15 (m, 20H, Ph), 5.81-5.71 (m, 1H, All), 5.49 (dd, 1H, *J*<sub>1,2</sub> = 1.7, *J*<sub>2,3</sub> = 3.2 Hz, H-2<sub>A</sub>), 5.20-5.08 (m, 2H, All), 4.90 (d, 1H, H-1<sub>A</sub>), 4.84-4.50 (m, 8H, PhCH<sub>2</sub>), 4.65 (d, 1H, *J*<sub>1,2</sub> < 1.0 Hz, H-1<sub>B</sub>), 4.04-3.85 (m, 2H, All), 4.02 (m, 2H, CH<sub>2</sub>Cl), 3.93 (dd, 1H, *J*<sub>2,3</sub> = 3.0 Hz, H-2<sub>B</sub>), 3.88 (dd, 1H, *J*<sub>3,4</sub> = 9.5 Hz, H-3<sub>A</sub>), 3.81 (pt, 1H, *J*<sub>3,4</sub> = 9.5 Hz, H-3<sub>B</sub>), 3.73 (dq, 1H, *J*<sub>4,5</sub> = 9.5, *J*<sub>5,6</sub> = 6.2 Hz, H-5<sub>A</sub>), 3.62 (dq, 1H, *J*<sub>4,5</sub> = 9.0, *J*<sub>5,6</sub> = 6.1 Hz, H-5<sub>B</sub>), 3.34 (dd, 1H, H-4<sub>B</sub>), 3.30 (dd, 1H, H-4<sub>A</sub>), 1.22 (d, 3H, H-6<sub>A</sub>), 1.21 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR:  $\delta$  166.9 (C=O), 138.5-117.2 (Ph, All), 99.2 (C-1<sub>A</sub>), 98.2 (C-1<sub>B</sub>), 80.4 (C-4<sub>A</sub>), 80.3 (C-3<sub>B</sub>), 80.2 (C-4<sub>B</sub>), 77.9 (C-3<sub>A</sub>), 75.8, 75.7, 72.6, 72.4 (4C, PhCH<sub>2</sub>), 74.9 (C-2<sub>B</sub>), 71.2 (C-2<sub>A</sub>), 68.6 (C-5<sub>A</sub>), 68.4 (C-5<sub>B</sub>), 68.0 (All), 41.3 (CH<sub>2</sub>Cl), 18.3 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>45</sub>H<sub>51</sub>ClO<sub>10</sub> (M, 786.3) *m/z* 809.3 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>45</sub>H<sub>51</sub>ClO<sub>10</sub>: C, 68.65; H, 6.53%. Found: C, 68.51; H, 6.67%.

**(3,4-Di-*O*-benzyl-2-*O*-chloroacetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha/\beta$ -L-rhamnopyranose (333).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (40 mg, 46  $\mu$ mol) was dissolved THF (7 mL), and the resulting red solution was processed as described for the preparation of 318. A solution of 332 (2.39 g, 3.04 mmol) in THF (18 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (30 mL) and water (5 mL). Mercuric chloride (1.24 g, 4.56 mmol) and mercuric oxide (1.3 g, 6.08 mmol) were added. The mixture, protected from light, was stirred for 2 h at rt, then concentrated. The residue was taken up in DCM and washed three times with satd aqueous KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (cyclohexane-EtOAc, 4:1) to give 333 (1.91 g, 84 %) as a white foam: [ $\alpha$ ]<sub>D</sub> -2° (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR:  $\delta$  7.40-7.10 (m, 20H, Ph), 5.49 (dd, 1H, *J*<sub>1,2</sub> = 1.7, *J*<sub>2,3</sub> = 3.2 Hz, H-2<sub>A</sub>), 4.99 (d, 1H, *J*<sub>1,2</sub> < 1.0 Hz,

H-1<sub>B</sub>), 4.90 (d, 1H, H-1<sub>A</sub>), 4.85-4.45 (m, 8H, PhCH<sub>2</sub>), 4.01 (m, 2H, CH<sub>2</sub>Cl), 3.93 (dd, 1H,  $J_{2,3} = 3.0$  Hz, H-2<sub>B</sub>), 3.90 (dd, 1H,  $J_{3,4} = 9.3$  Hz, H-3<sub>A</sub>), 3.84 (dd, 1H,  $J_{3,4} = 9.0$  Hz, H-3<sub>B</sub>), 3.81 (dq, 1H,  $J_{4,5} = 9.0$  Hz,  $J_{5,6} = 6.2$  Hz, H-5<sub>B</sub>), 3.72 (dq, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.2$  Hz, H-5<sub>A</sub>), 3.33 (pt, 1H, H-4<sub>B</sub>), 3.30 (dd, 1H, H-4<sub>A</sub>), 2.81 (d, 1H,  $J_{2,OH} = 3.4$  Hz, OH), 1.22 (d, 3H, H-6<sub>A</sub>), 1.20 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR: δ 167.0 (C=O), 138.5-127.2 (Ph), 99.1 (C-1<sub>A</sub>), 93.9 (C-1<sub>B</sub>), 80.3 (C-4<sub>B</sub>), 80.2 (C-4<sub>A</sub>), 79.7 (C-3<sub>B</sub>), 77.8 (C-3<sub>A</sub>), 75.8, 75.7, 72.6, 72.4 (4C, PhCH<sub>2</sub>), 75.0 (C-2<sub>B</sub>), 71.1 (C-2<sub>A</sub>), 68.6 (C-5<sub>A</sub>), 68.4 (C-5<sub>B</sub>), 41.3 (CH<sub>2</sub>Cl), 18.1 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>42</sub>H<sub>47</sub>ClO<sub>10</sub> (M, 746.3)  $m/z$  769.3 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>42</sub>H<sub>47</sub>ClO<sub>10</sub>: C, 67.51; H, 6.34%. Found: C, 67.46; H, 6.39%.

**(3,4-Di-*O*-benzyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl Trichloroacetimidate (334).** The hemiacetal 333 (1.80 g, 2.41 mmol) was dissolved in DCM (25 mL), placed under Argon and cooled to 0°C. Trichloroacetonitrile (2.4 mL, 24 mmol), then DBU (35 μL, 0.24 mmol) were added. The mixture was stirred at 0°C for 40 min. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was eluted from a column of silica gel with 4:1 cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give 334 (1.78 g, 83 %) as a colorless foam: [α]<sub>D</sub> -12° (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR: δ 8.60 (s, 1H, NH), 7.50-7.30 (m, 20H, Ph), 6.21 (d, 1H,  $J_{1,2} = 1.8$  Hz, H-1<sub>B</sub>), 5.63 (dd, 1H,  $J_{1,2} = 1.5$ ,  $J_{2,3} = 3.2$  Hz, H-2<sub>A</sub>), 5.07 (d, 1H, H-1<sub>A</sub>), 5.00-4.65 (m, 8H, PhCH<sub>2</sub>), 4.19 (m, 2H, CH<sub>2</sub>Cl), 4.09 (dd, 1H,  $J_{2,3} = 3.2$  Hz, H-2<sub>B</sub>), 4.04 (dd, 1H,  $J_{3,4} = 9.0$  Hz, H-3<sub>B</sub>), 3.95 (m, 3H, H-3<sub>A</sub>, 5<sub>A</sub>, 5<sub>B</sub>), 3.58 (dd, 1H, H-4<sub>A</sub>), 3.48 (dd, 1H, H-4<sub>B</sub>), 1.39 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR: δ 167.1 (C=O), 160.7 (C=N), 138.3-127.0 (Ph), 99.4 (C-1<sub>A</sub>), 97.5 (C-1<sub>B</sub>), 91.4 (CCl<sub>3</sub>), 80.1 (C-4<sub>B</sub>), 80.0 (C-4<sub>A</sub>), 79.2 (C-3<sub>A</sub>), 77.9 (C-3<sub>B</sub>), 75.9, 75.8, 73.0, 72.6 (4C, PhCH<sub>2</sub>), 73.7 (C-2<sub>B</sub>), 71.4 (C-2<sub>A</sub>), 71.2, 68.9 (2C, C-5<sub>A</sub>, 5<sub>B</sub>), 41.3 (CH<sub>2</sub>Cl), 18.4, 18.2 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). Anal. Calcd for C<sub>44</sub>H<sub>47</sub>Cl<sub>4</sub>NO<sub>10</sub>: C, 59.27; H, 5.31; N, 1.57%. Found: C, 59.09; H, 5.49; N, 1.53%.

**Allyl (3,4-Di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (335).** The alcohol 323 (3.8 g, 5.35 mmol) was dissolved in DMF (25 mL). The mixture was cooled to 0°C and NaH (320 mg, 8.02 mmol) was added in 3 parts each 10 min. Then *p*MeOBnCl (1.8 mL, 13.34 mmol) was added and the mixture was stirred overnight at rt. MeOH (5 mL) was added and the solution stirred for 10 min. The solution was concentrated and the residue was eluted from a column of silica gel with 95:5 cyclohexane-acetone to give 335 (4.34 g, 97 %) as a colorless syrup: [α]<sub>D</sub> -8° (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz): δ 7.20-6.80 (m, 24H, Ph), 5.90-5.80 (m, 1H, All), 5.30-5.15 (m, 2H, All), 5.12 (d, 1H,  $J_{1,2} < 1.0$  Hz, H-1<sub>A</sub>), 4.73 (d, 1H,  $J_{1,2} < 1.0$  Hz, H-1<sub>B</sub>), 4.70-4.40 (m, 10H, PhCH<sub>2</sub>), 4.20-4.08 (m, 1H, All), 4.10 (dd, 1H,  $J_{2,3} = 3.0$  Hz, H-2<sub>B</sub>), 3.95-3.88 (m, 3H, H-3<sub>A</sub>, 3<sub>B</sub>, All), 3.80-3.78 (m, 2H,  $J_{4,5} = 9.4$ ,  $J_{5,6} = 6.1$  Hz, H-2<sub>A</sub>, 5<sub>A</sub>), 3.72 (s, 3H, OCH<sub>3</sub>), 3.70 (m, 1H,  $J_{4,5} = 9.4$ ,  $J_{5,6} = 6.1$  Hz, H-5<sub>B</sub>), 3.61 (dd, 1H, H-4<sub>A</sub>), 3.32 (dd, 1H, H-4<sub>B</sub>), 1.18 (d, 3H, H-6<sub>A</sub>), 1.10 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR (75

MHz):  $\delta$  133.9-113.8 (Ph, All), 99.0 (C-1<sub>A</sub>), 97.8 (C-1<sub>B</sub>), 80.4 (C-4<sub>A</sub>), 80.2 (C-4<sub>B</sub>), 80.0 (C-3<sub>B</sub>), 79.0 (C-3<sub>A</sub>), 75.2, 72.3, 71.8, 71.5, 71.3, 67.5 (5C, PhCH<sub>2</sub>, All), 74.1 (C-2<sub>A</sub>), 73.8 (C-2<sub>B</sub>), 68.3 (C-5<sub>A</sub>), 67.8 (C-5<sub>B</sub>), 55.0 (OCH<sub>3</sub>), 17.8, 17.9 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>51</sub>H<sub>58</sub>O<sub>10</sub> (M, 830.4)  $m/z$  853.5 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>51</sub>H<sub>58</sub>O<sub>10</sub>: C, 73.71; H, 7.03%. Found: C, 73.57; H, 7.21%.

**(3,4-Di-*O*-benzyl-2-*O*-*p*-methoxybenzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranose (336).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (50 mg, 60  $\mu$ mol) was dissolved in THF (6 mL), and the resulting red solution was processed as described for the preparation of 318. A solution of 335 (4.23 g, 5.09 mmol) in THF (24 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated. The residue was taken up in acetone (45 mL), and water (5 mL) was added. Mercuric chloride (2.07 g, 7.63 mmol) and mercuric oxide (2.2 g, 10.2 mmol) were added. The mixture, protected from light, was stirred for 2 h at rt, then concentrated. The residue was taken up in DCM and washed three times with satd aqueous KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (cyclohexane-EtOAc, 4:1) to give 336 (2.97 g, 73 %) as a white foam:  $[\alpha]_D^{+8^\circ}$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz):  $\delta$  7.40-7.25 (m, 20H, Ph), 7.18-6.73 (m, 4H, Ph), 5.12 (d, 1H,  $J_{1,2} < 1.0$  Hz, H-1<sub>A</sub>), 5.05 (d, 1H,  $J_{1,2} < 1.0$  Hz, H-1<sub>B</sub>), 4.80-4.40 (m, 10H, PhCH<sub>2</sub>), 4.08 (dd, 1H,  $J_{2,3} = 3.0$  Hz, H-2<sub>B</sub>), 3.90-3.80 (m, 2H,  $J_{3,4} = J_{4,5} = 9.5$ ,  $J_{5,6} = 6.1$  Hz, H-3<sub>B</sub>, 5<sub>B</sub>), 3.80-3.78 (m, 2H,  $J_{2,3} = 3.1$ ,  $J_{4,5} = 9.4$ ,  $J_{5,6} = 6.1$  Hz, H-2<sub>A</sub>, 5<sub>A</sub>), 3.73 (m, 1H,  $J_{3,4} = 9.4$  Hz, H-3<sub>A</sub>), 3.72 (s, 3H, OCH<sub>3</sub>), 3.60 (pt, 1H, H-4<sub>A</sub>), 3.33 (pt, 1H, H-4<sub>B</sub>), 1.34 (d, 3H, H-6<sub>A</sub>), 1.24 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR (75 MHz):  $\delta$  113.2-129.8 (Ph), 99.1 (C-1<sub>A</sub>), 93.8 (C-1<sub>B</sub>), 80.7 (C-4<sub>A</sub>), 80.3 (C-4<sub>B</sub>), 79.7 (C-3<sub>B</sub>), 79.2 (C-3<sub>A</sub>), 75.5, 75.4, 72.6, 72.5, 72.4 (5C, PhCH<sub>2</sub>), 74.2 (C-2<sub>A</sub>), 74.1 (C-2<sub>B</sub>), 68.5 (C-5<sub>A</sub>), 68.1 (C-5<sub>B</sub>), 55.3 (OCH<sub>3</sub>), 18.1 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). FAB-MS for C<sub>48</sub>H<sub>54</sub>O<sub>10</sub> (M, 790.4)  $m/z$  813.4 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>48</sub>H<sub>54</sub>O<sub>10</sub>: C, 72.89; H, 6.88%. Found: C, 72.86; H, 6.98%.

**(3,4-Di-*O*-benzyl-2-*O*-*p*-methoxybenzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha/\beta$ -L-rhamnopyranosyl Trichloroacetimidate (337).** The hemiacetal 336 (2.1 g, 2.66 mmol) was dissolved in DCM (20 mL), placed under Argon and cooled to 0°C. Trichloroacetonitrile (2.7 mL, 26 mmol), then DBU (40  $\mu$ L, 0.26 mmol) were added. The mixture was stirred at 0°C for 30 min. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was eluted from a column of silica gel with 8:2 cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give 337 (2.03 g, 82 %) as a colorless foam:  $[\alpha]_D^{-10^\circ}$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz):  $\delta$  8.50 (s, 1H, NH), 7.25-7.05 (m, 20H, Ph), 7.05-6.62 (m, 4H, Ph), 6.08 (d, 1H,  $J_{1,2} < 1.0$  Hz, H-1<sub>B</sub>), 5.10 (d, 1H,  $J_{1,2} < 1.0$  Hz, H-1<sub>A</sub>), 4.80-4.40 (m, 10H, PhCH<sub>2</sub>), 4.10 (dd, 1H,  $J_{2,3} = 3.0$  Hz, H-2<sub>B</sub>), 3.90-3.80 (m, 4H, H-3<sub>B</sub>, 2<sub>A</sub>, 3<sub>A</sub>, 5<sub>A</sub>), 3.80-3.72 (m, 1H, H-5<sub>B</sub>), 3.72 (s, 3H, OCH<sub>3</sub>), 3.63 (pt,

1H,  $J_{3,4} = J_{4,5} = 9.5$  Hz, H-4<sub>A</sub>), 3.42 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.5$  Hz, H-4<sub>B</sub>), 1.30 (d, 3H, H-6<sub>B</sub>), 1.25 (d, 3H, H-6<sub>A</sub>). <sup>13</sup>C NMR (75 MHz):  $\delta$  161.1 (C=NH), 129.5-113.4 (Ph), 99.6 (C-1<sub>A</sub>), 97.0 (C-1<sub>B</sub>), 80.6 (C-4<sub>A</sub>), 79.6 (C-4<sub>B</sub>), 79.3 (2C, C-3<sub>A</sub>, 3<sub>B</sub>), 75.7, 75.5, 72.8, 72.3, 72.0 (5C, PhCH<sub>2</sub>), 74.4 (C-2<sub>A</sub>), 72.6 (C-2<sub>B</sub>), 71.1 (C-5<sub>A</sub>), 68.9 (C-5<sub>B</sub>), 55.3 (OCH<sub>3</sub>), 18.1 (2C, C-6<sub>A</sub>, 6<sub>B</sub>). Anal. Calcd. for C<sub>50</sub>H<sub>54</sub>Cl<sub>3</sub>NO<sub>10</sub>: C, 64.21; H, 5.82; N, 1.50%. Found: C, 64.67; H, 6.01; N, 1.28%.

**Allyl (3,4-Di-*O*-benzyl-2-*O*-chloroacetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (338).** A mixture of alcohol 311 (212 mg, 0.255 mmol) and imidate 334 (270 mg, 0.33 mmol) in anhydrous Et<sub>2</sub>O (4 mL) was stirred for 15 min under dry Argon. After cooling at -60°C, TMSOTf (30  $\mu$ L, 0.166 mmol) was added dropwise and the mixture was stirred overnight and allowed to reach rt. Triethylamine (120  $\mu$ L) was added and the mixture was concentrated. The residue was eluted from a column of silica gel with 7:1 cyclohexane-EtOAc to give 338 (86 mg, 22 %) as a foam:  $[\alpha]_D^{+5^\circ}$  (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (300 MHz)  $\delta$  8.00-6.95 (m, 45H, Ph), 6.00-5.80 (m, 1H, All), 5.56 (dd, 1H, H-2<sub>A</sub>), 5.40 (dd, 1H,  $J_{1,2} < 1.0$ ,  $J_{2,3} = 3.0$  Hz, H-2<sub>C</sub>), 5.37-5.20 (m, 2H, All), 5.08 (d, 1H,  $J_{1,2} = 3.2$  Hz, H-1<sub>E</sub>), 5.04 (d, 1H,  $J_{1,2} < 1.0$  Hz, H-1<sub>A</sub>), 5.00 (d, 1H,  $J_{1,2} < 1.0$  Hz, H-1<sub>B</sub>), 4.99 (d, 1H, H-1<sub>C</sub>), 4.90-4.30 (m, 16H, CH<sub>2</sub>Ph), 4.35 (dd, 1H,  $J_{2,3} = 3.0$  Hz, H-2<sub>B</sub>), 4.14 (dd, 1H,  $J_{3,4} = 9.5$  Hz, H-3<sub>C</sub>), 4.03 (pt, 1H,  $J_{2,3} = J_{3,4} = 10.0$  Hz, H-3<sub>E</sub>), 4.20-3.90 (m, 2H, All), 4.00-3.75 (m, 4H, CH<sub>2</sub>Cl, H-6a<sub>E</sub>, 6b<sub>E</sub>), 3.96 (dd, 1H, H-3<sub>A</sub>), 3.95 (m, 1H, H-5<sub>A</sub>), 3.95 (m, 1H, H-5<sub>E</sub>), 3.83 (dd, 1H, H-4<sub>C</sub>), 3.80 (m, 1H, H-5<sub>C</sub>), 3.72 (dd, 1H, H-4<sub>E</sub>), 3.64 (dd, 1H, H-3<sub>B</sub>), 3.60 (m, 1H, H-5<sub>B</sub>), 3.52 (dd, 1H, H-2<sub>E</sub>), 3.39 (dd, 1H, H-4<sub>A</sub>), 3.30 (dd, 1H, H-4<sub>B</sub>), 1.35 (d, 1H, H-6<sub>A</sub>), 1.30 (d, 1H, H-6<sub>C</sub>), 1.00 (d, 1H, H-6<sub>B</sub>); <sup>13</sup>C NMR (75 MHz)  $\delta$  166.1, 165.7 (C=O), 133.4-117.0 (Ph), 100.9 (C-1<sub>B</sub>), 98.9 (C-1<sub>A</sub>), 97.8 (C-1<sub>E</sub>), 96.0 (C-1<sub>C</sub>), 81.8 (C-3<sub>E</sub>), 80.9 (C-2<sub>E</sub>), 79.9 (C-4<sub>A</sub>), 79.6 (C-4<sub>B</sub>), 79.6 (C-3<sub>C</sub>), 78.9 (C-3<sub>B</sub>), 78.0 (C-4<sub>C</sub>), 77.5 (C-4<sub>E</sub>), 77.3 (C-3<sub>A</sub>), 75.6, 75.3, 75.0, 74.7, 73.9, 73.5, 72.8, 70.9 (9C, CH<sub>2</sub>Ph, All), 74.9 (C-2<sub>B</sub>), 72.5 (C-2<sub>C</sub>), 71.2 (C-5<sub>E</sub>), 70.9 (C-2<sub>A</sub>), 68.8 (C-5<sub>B</sub>), 68.5 (C-6<sub>E</sub>), 68.3 (C-5<sub>A</sub>), 67.5 (C-5<sub>C</sub>), 40.9 (CH<sub>2</sub>Cl), 18.8 (C-6<sub>A</sub>), 18.2 (C-6<sub>C</sub>), 17.8 (C-6<sub>B</sub>). FAB-MS for C<sub>92</sub>H<sub>99</sub>ClO<sub>20</sub> (M, 1558.6)  $m/z$  1581.7 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>92</sub>H<sub>99</sub>ClO<sub>20</sub>: C, 70.82; H, 6.40%. Found: C, 70.67; H, 6.58%.

**Allyl (3,4-Di-*O*-benzyl-2-*O*-*p*-methoxybenzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (339).** A mixture of alcohol 311 (125 mg, 0.15 mmol) and 4Å molecular sieves in anhydrous Et<sub>2</sub>O (3 mL) was stirred for 45 min under dry Argon. After cooling at -40°C, Me<sub>3</sub>SiOTf (20  $\mu$ L, 0.112 mmol) was added dropwise. A solution of the donor 337 (210 mg, 0.225 mmol) in anhydrous Et<sub>2</sub>O (2 mL) was added dropwise to the solution of the acceptor during 1 h. The mixture was stirred for 3 h at -40°C. Triethylamine (100  $\mu$ L) was added and the mixture

was filtered and concentrated. The residue was eluted from a column of silica gel with 85:15 cyclohexane-EtOAc to give **339** (107 mg, 44 %) as a foam:  $[\alpha]_D +12^\circ$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR:  $\delta$  8.10-7.10 (m, 45H, Ph), 7.00-6.50 (m, 4H, CH<sub>2</sub>PhOMe), 5.90-5.70 (m, 1H, All), 5.32 (dd, 1H,  $J_{1,2} = 1.6$ ,  $J_{2,3} = 3.1$  Hz, H-2<sub>C</sub>), 5.25-5.10 (m, 2H, All), 5.05 (d, 1H, H-1<sub>B</sub>), 4.98 (d, 1H,  $J_{1,2} = 3.2$  Hz, H-1<sub>E</sub>), 4.85 (m, 2H, H-1<sub>A</sub>, 1<sub>C</sub>), 4.80-4.20 (m, 18H, CH<sub>2</sub>Ph), 4.20-3.90 (m, 2H, All), 4.20-3.00 (m, 20H, H-2<sub>A</sub>, 2<sub>B</sub>, 2<sub>E</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>E</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>E</sub>, 6<sub>A</sub><sub>E</sub>, 6<sub>B</sub><sub>E</sub>, OCH<sub>3</sub>), 1.30-0.82 (3 d, 9H, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>); <sup>13</sup>C NMR:  $\delta$  166.3 (C=O), 138.5-118.2 (Ph, All), 99.5, 99.3 (2C, C-1<sub>A</sub>, 1<sub>B</sub>), 98.4 (C-1<sub>E</sub>), 96.4 (C-1<sub>C</sub>), 82.3, 81.4, 81.1, 80.5, 80.3, 79.5, 78.2, 77.6 (8C, C-2<sub>E</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>), 76.0, 75.5, 75.3, 74.9, 74.3, 73.3, 72.3, 71.8, 71.6 (9C, CH<sub>2</sub>Ph), 74.1, 73.8 (2C, C-2<sub>A</sub>, 2<sub>B</sub>), 72.5 (C-2<sub>C</sub>), 72.0 (C-4<sub>E</sub>), 69.2, 69.0, 68.9 (3C, C-5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>), 68.8, 68.6 (All, C-6<sub>E</sub>), 67.8 (C-5<sub>E</sub>), 55.5 (OCH<sub>3</sub>), 19.0, 18.8, 18.4 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). FAB-MS for C<sub>98</sub>H<sub>106</sub>O<sub>20</sub> (M, 1603.8) *m/z* 1626.6 [M + Na]<sup>+</sup>.

**Allyl (3,4-Di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (310).** A solution of the trisaccharide **342** (Segat, F.; Mulard, L. A. *Tetrahedron: Asymmetry* **2002**, *13*, 2211-2222) (8.0 g, 6.5 mmol) in MeOH (128 mL) was treated with 5.7 mL of HBF<sub>4</sub>/Et<sub>2</sub>O at rt. The solution was stirred during 4 days. Et<sub>3</sub>N was added until neutralization and concentrated. The residue was diluted with DCM, washed with satd aq NaHCO<sub>3</sub> and water. The organic layer was dried on MgSO<sub>4</sub>, filtered and concentrated. The residue was eluted from a column of silica gel with 15:1 toluene-EtOAc to give **310** (6.31 g, 84 %) as a foam:  $[\alpha]_D +14^\circ$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR:  $\delta$  8.10-7.05 (m, 35H, Ph), 5.82 (m, 1H, All), 5.25 (dd, 1H,  $J_{1,2} = 1.7$ ,  $J_{2,3} = 3.1$  Hz, H-2<sub>C</sub>), 5.19 (m, 2H, All), 5.00 (d, 1H,  $J_{1,2} = 3.1$  Hz, H-1<sub>E</sub>), 4.87 (d, 1H,  $J_{1,2} = 1.8$  Hz, H-1<sub>B</sub>), 4.81 (d, 1H, H-1<sub>C</sub>), 4.90-4.35 (m, 12H, CH<sub>2</sub>Ph), 4.20-4.00 (m, 2H, All), 4.10 (dd, 1H,  $J_{3,4} = 8.5$  Hz, H-3<sub>C</sub>), 4.09 (dd, 1H,  $J_{2,3} = 3.2$  Hz, H-2<sub>B</sub>), 3.95 (m, 1H,  $J_{4,5} = 9.5$  Hz, H-5<sub>E</sub>), 3.92 (pt, 1H,  $J_{2,3} = 9.5 = J_{3,4} = 9.5$  Hz, H-3<sub>E</sub>), 3.78 (dq, 1H,  $J_{5,6} = 6.0$  Hz, H-5<sub>C</sub>), 3.70 (m, 1H, H-4<sub>C</sub>), 3.62-3.58 (m, 2H, H-6<sub>A</sub><sub>E</sub>, 6<sub>B</sub><sub>E</sub>), 3.59 (m, 1H,  $J_{4,5} = 9.0$ ,  $J_{5,6} = 6.2$  Hz, H-5<sub>B</sub>), 3.54 (dd, 1H, H-4<sub>E</sub>), 3.48 (dd, 1H,  $J_{3,4} = 8.5$  Hz, H-3<sub>B</sub>), 3.45 (dd, 1H, H-2<sub>E</sub>), 3.31 (dd, 1H, H-4<sub>B</sub>), 2.68 (d, 1H,  $J_{2,OH} = 2.3$  Hz, OH), 1.29 (d, 3H, H-6<sub>C</sub>), 1.09 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR:  $\delta$  166.2 (C=O), 137.5-118.2 (Ph, All), 103.1 (C-1<sub>B</sub>), 98.5 (C-1<sub>E</sub>), 96.6 (C-1<sub>C</sub>), 82.1 (C-3<sub>E</sub>), 81.4 (C-2<sub>E</sub>), 80.4 (C-4<sub>B</sub>), 79.7 (C-3<sub>B</sub>), 79.4 (C-4<sub>C</sub>), 78.9 (C-3<sub>C</sub>), 78.1 (C-4<sub>E</sub>), 76.0, 75.5, 74.5, 74.2, 73.6, 72.1 (6C, CH<sub>2</sub>Ph), 73.7 (C-2<sub>C</sub>), 71.6 (C-2<sub>B</sub>), 68.9 (C-6<sub>E</sub>), 68.8 (C-5<sub>B</sub>), 68.7 (All, C-5<sub>E</sub>), 68.1 (C-5<sub>C</sub>), 19.1 (C-6<sub>C</sub>), 18.2 (C-6<sub>B</sub>). FAB-MS for C<sub>70</sub>H<sub>76</sub>O<sub>15</sub> (M, 1156.5) *m/z* 1179.5 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>70</sub>H<sub>76</sub>O<sub>15</sub>: C, 72.64; H, 6.62%. Found: C, 72.49; H, 6.80%.

**Allyl (2-*O*-Acetyl-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (344).** A mixture of

alcohol **310** (5.2 g, 4.49 mmol), imidate **321** (3.58 g, 6.74 mmol) and 4 Å molecular sieves in anhydrous Et<sub>2</sub>O (117 mL) was stirred for 1 h under dry Argon. After cooling at -30°C, Me<sub>3</sub>SiOTf (580 µL, 3.2 mmol) was added dropwise and the mixture was stirred and allowed to rt overnight. Triethylamine (1.2 mL) was added and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel with 9:1 cyclohexane-EtOAc to give **344** (6.16 g, 90 %) as a white foam:  $[\alpha]_D^{+13}$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR: δ 8.10-7.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.45 (dd, 1H, *J*<sub>1,2</sub> = 1.5, *J*<sub>2,3</sub> = 2.5 Hz, H-2<sub>A</sub>), 5.29 (dd, 1H, *J*<sub>1,2</sub> = 1.5, *J*<sub>2,3</sub> = 2.5 Hz, H-2<sub>C</sub>), 5.19 (m, 2H, All), 4.97 (d, 1H, *J*<sub>1,2</sub> = 3.2 Hz, H-1<sub>E</sub>), 4.95 (d, 1H, H-1<sub>A</sub>), 4.91 (d, 1H, *J*<sub>1,2</sub> = 1.6 Hz, H-1<sub>B</sub>), 4.84 (d, 1H, H-1<sub>C</sub>), 4.90-4.35 (m, 16H, CH<sub>2</sub>Ph), 4.29 (dd, 1H, *J*<sub>2,3</sub> = 2.6 Hz, H-2<sub>B</sub>), 4.10-4.00 (m, 2H, All), 4.02 (dd, 1H, *J*<sub>3,4</sub> = 8.5 Hz, H-3<sub>C</sub>), 3.90 (m, 2H, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = *J*<sub>4,5</sub> = 9.5 Hz, H-3<sub>E</sub>, 5<sub>E</sub>), 3.85 (m, 2H, *J*<sub>3,4</sub> = 9.3, *J*<sub>4,5</sub> = 9.5 Hz, H-3<sub>A</sub>, 5<sub>A</sub>), 3.72 (m, 2H, *J*<sub>5,6</sub> = 6.0 Hz, H-4<sub>C</sub>, 5<sub>C</sub>), 3.66-3.62 (m, 2H, H-6<sub>A</sub><sub>E</sub>, 6<sub>B</sub><sub>E</sub>), 3.61 (dd, 1H, H-4<sub>E</sub>), 3.54 (dd, 1H, *J*<sub>3,4</sub> = 9.4 Hz, H-3<sub>B</sub>), 3.45 (dd, 1H, *J*<sub>4,5</sub> = 9.5, *J*<sub>5,6</sub> = 6.1 Hz, H-5<sub>B</sub>), 3.39 (dd, 1H, H-2<sub>E</sub>), 3.34 (dd, 1H, H-4<sub>A</sub>), 3.21 (dd, 1H, H-4<sub>B</sub>), 1.89 (s, 3H, OAc), 1.26 (2d, 6H, H-6<sub>A</sub>, 6<sub>C</sub>), 0.89 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR: δ 170.2, 166.1 (C=O), 138.4-118.1 (Ph, All), 101.3 (C-1<sub>B</sub>), 99.8 (C-1<sub>A</sub>), 98.2 (C-1<sub>E</sub>), 96.4 (C-1<sub>C</sub>), 82.2 (C-3<sub>E</sub>), 81.4 (C-2<sub>E</sub>), 80.6 (C-4<sub>A</sub>), 80.5 (C-3<sub>C</sub>), 80.1 (C-4<sub>B</sub>), 79.3 (C-3<sub>B</sub>), 78.5 (C-4<sub>C</sub>), 78.1 (C-3<sub>A</sub>), 78.0 (C-4<sub>E</sub>), 76.0, 75.9, 75.7, 75.2, 74.3, 73.3, 72.1, 71.1 (8C, CH<sub>2</sub>Ph), 75.2 (C-2<sub>B</sub>), 72.9 (C-2<sub>C</sub>), 71.7 (C-5<sub>E</sub>), 69.5 (C-2<sub>A</sub>), 69.2 (2C, C-5<sub>A</sub>, 5<sub>B</sub>), 68.9 (All), 68.9 (C-6<sub>E</sub>), 67.9 (C-5<sub>C</sub>), 21.4 (OAc), 19.1 (C-6<sub>A</sub>), 18.7 (C-6<sub>C</sub>), 18.1 (C-6<sub>B</sub>). FAB-MS for C<sub>90</sub>H<sub>100</sub>O<sub>20</sub> (M, 1524.7) *m/z* 1547.8 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>92</sub>H<sub>100</sub>O<sub>20</sub>: C, 72.42; H, 6.61%. Found: C, 72.31; H, 6.75%.

**Allyl (3,4-Di-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl-(1→4)]-2-O-benzoyl-α-L-rhamnopyranoside (340).** A mixture of **344** (6.0 g, 3.93 mmol) in MeOH (200 mL) was treated with 10 mL of HBF<sub>4</sub>/Et<sub>2</sub>O at rt. The solution was stirred during 5 days. Et<sub>3</sub>N was added until neutralization and concentrated. The residue was diluted with DCM, washed with satd aq NaHCO<sub>3</sub> and water. The organic layer was dried on MgSO<sub>4</sub>, filtered and concentrated. The residue was eluted from a column of silica gel with 6:1 cyclohexane-EtOAc to give **340** (5.0 g, 84 %) as a colourless foam:  $[\alpha]_D^{+12}$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR: δ 8.00-7.00 (m, 45H, Ph), 5.83 (m, 1H, All), 5.29 (dd, 1H, *J*<sub>1,2</sub> = 1.8, *J*<sub>2,3</sub> = 2.9 Hz, H-2<sub>C</sub>), 5.19 (m, 2H, All), 4.99 (d, 1H, *J*<sub>1,2</sub> = 1.4 Hz, H-1<sub>A</sub>), 4.97 (d, 1H, *J*<sub>1,2</sub> = 3.3 Hz, H-1<sub>E</sub>), 4.94 (d, 1H, *J*<sub>1,2</sub> = 1.7 Hz, H-1<sub>B</sub>), 4.83 (d, 1H, H-1<sub>C</sub>), 4.90-4.35 (m, 16H, CH<sub>2</sub>Ph), 4.30 (dd, 1H, *J*<sub>2,3</sub> = 2.7 Hz, H-2<sub>B</sub>), 4.10-4.00 (m, 2H, All), 4.02 (dd, 1H, *J*<sub>2,3</sub> = 3.5, *J*<sub>3,4</sub> = 8.5 Hz, H-3<sub>C</sub>), 3.98 (m, 1H, H-2<sub>A</sub>), 3.95-3.91 (m, 3H, H-5<sub>E</sub>, 6<sub>A</sub><sub>E</sub>, 6<sub>B</sub><sub>E</sub>), 3.90 (dd, 1H, *J*<sub>2,3</sub> = 9.5, *J*<sub>3,4</sub> = 9.4 Hz, H-3<sub>E</sub>), 3.82-3.73 (m, 4H, H-3<sub>A</sub>, 5<sub>A</sub>, 4<sub>C</sub>, 5<sub>C</sub>), 3.66 (dd, 1H, *J*<sub>4,5</sub> = 9.6 Hz, H-4<sub>E</sub>), 3.53 (dd, 1H, *J*<sub>3,4</sub> = 9.5 Hz, H-3<sub>B</sub>), 3.48 (m, 1H, *J*<sub>4,5</sub> = 9.5 Hz, H-5<sub>B</sub>), 3.44-3.40 (m, 2H, H-4<sub>A</sub>, 2<sub>E</sub>), 3.17 (pt, 1H, H-4<sub>B</sub>), 2.18 (d, 1H, *J*<sub>2,OH</sub> = 2.0 Hz, OH), 1.26 (d, 3H,



$J_{5,6} = 5.5$  Hz, H-6<sub>C</sub>), 1.25 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>A</sub>), 0.90 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>B</sub>); <sup>13</sup>C NMR: δ 166.2 (C=O), 138.3-118.0 (Ph, All), 101.5 (C-1<sub>B</sub>), 101.4 (C-1<sub>A</sub>), 98.2 (C-1<sub>E</sub>), 96.4 (C-1<sub>C</sub>), 82.2 (C-3<sub>E</sub>), 81.4 (C-2<sub>E</sub>), 80.6 (C-4<sub>A</sub>), 80.3 (C-4<sub>B</sub>), 79.9 (2C, C-3<sub>C</sub>, 3<sub>A</sub>), 79.2 (C-3<sub>B</sub>), 78.3 (C-4<sub>C</sub>), 78.0 (C-4<sub>E</sub>), 75.9, 75.6, 75.5, 74.8, 74.2, 73.5, 72.4, 71.0 (8C, CH<sub>2</sub>Ph), 75.3 (C-2<sub>B</sub>), 72.9 (C-2<sub>C</sub>), 71.6 (C-2<sub>A</sub>), 69.2, 69.1, 68.3, 67.9 (4C, C-5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>E</sub>), 68.9, 68.7 (3C, C-6<sub>D</sub>, 6<sub>E</sub>, All), 19.1 (C-6<sub>C</sub>), 18.6 (C-6<sub>A</sub>), 18.1 (C-6<sub>B</sub>). FAB-MS for C<sub>90</sub>H<sub>98</sub>O<sub>19</sub> (M, 1482.7)  $m/z$  1505.8 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>90</sub>H<sub>98</sub>O<sub>19</sub>·2H<sub>2</sub>O: C, 71.12; H, 6.77%. Found: C, 71.21; H, 6.78%.

**Allyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (304).** (a) A mixture of the donor **308** (200 mg, 230 μmol) and the acceptor **310** (188 mg, 144 μmol), 4 Å molecular sieves and dry Et<sub>2</sub>O:1,2-DCE (1:1, 5 mL) was stirred for 1.5 h then cooled to 0°C. NIS (104 mg, 0.46 mmol) and triflic acid (4 μL, 0.05 mmol) were successively added. The stirred mixture was allowed to reach rt in 1 h. Et<sub>3</sub>N (25 μL) was added and the mixture filtered. After evaporation, the residue was eluted from a column of silica gel with 4:1 to 2:1 cyclohexane-EtOAc to give **304** (28 mg, 10 %).

(b) A mixture of alcohol **310** (5.0 g, 3.37 mmol), imidate **316** (3.0 g, 5.04 mmol) and 4 Å molecular sieves in anhydrous DCM (120 mL) was stirred for 1 h under dry Argon. After cooling at 0°C, TMSOTf (240 μL, 1.32 mmol) was added dropwise and the mixture was stirred for 2.5 h while coming back to rt. Et<sub>3</sub>N (800 μL) was added, and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel with 4:1 to 2:1 cyclohexane-EtOAc to give **304** (6.27 g, 98 %) as a colourless foam: [α]<sub>D</sub> +1.5° (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR: δ 8.00-7.00 (m, 45H, Ph), 6.68 (d, 1H,  $J_{2,NH} = 8.5$  Hz, NH<sub>D</sub>), 5.82 (m, 1H, All), 5.29 (dd, 1H,  $J_{1,2} = 1.0$ ,  $J_{2,3} = 2.3$  Hz, H-2<sub>C</sub>), 5.19 (m, 2H, All), 5.00 (d, 1H,  $J_{1,2} = 1.0$  Hz, H-1<sub>A</sub>), 4.96 (dd, 1H,  $J_{2,3} = 10.5$ ,  $J_{3,4} = 10.5$  Hz, H-3<sub>D</sub>), 4.88 (d, 1H,  $J_{1,2} = 3.3$  Hz, H-1<sub>E</sub>), 4.85 (d, 1H, H-1<sub>C</sub>), 4.82 (d, 1H,  $J_{1,2} = 1.7$  Hz, H-1<sub>B</sub>), 4.81 (dd, 1H,  $J_{4,5} = 10.0$  Hz, H-4<sub>D</sub>), 4.72 (d, 1H,  $J_{1,2} = 8.6$  Hz, H-1<sub>D</sub>), 4.90-4.35 (m, 16H, CH<sub>2</sub>Ph), 4.38 (m, 1H, H-2<sub>B</sub>), 4.10-4.00 (m, 2H, All), 4.05 (dd, 1H,  $J_{2,3} = 2.7$  Hz, H-2<sub>A</sub>), 3.95 (dd, 1H,  $J_{2,3} = 3.5$ ,  $J_{3,4} = 8.5$  Hz, H-3<sub>C</sub>), 3.90 (m, 2H, H-5<sub>E</sub>, 4<sub>E</sub>), 3.86-3.82 (m, 2H, H-6a<sub>D</sub>, 6b<sub>D</sub>), 3.84-3.70 (m, 6H, H-3<sub>E</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 3<sub>A</sub>, 5<sub>A</sub>, 2<sub>D</sub>), 3.68 (m, 1H, H-5<sub>C</sub>), 3.61 (dd, 1H,  $J_{4,5} = 9.0$  Hz, H-4<sub>C</sub>), 3.56 (dd, 1H,  $J_{3,4} = 9.5$  Hz, H-3<sub>B</sub>), 3.47 (m, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.1$  Hz, H-5<sub>B</sub>), 3.35-3.33 (m, 3H, H-4<sub>A</sub>, 5<sub>D</sub>, 2<sub>E</sub>), 3.17 (dd, 1H, H-4<sub>B</sub>), 2.02, 2.00, 1.98 (3s, 9H, OAc), 1.24 (d, 3H,  $J_{5,6} = 6.0$  Hz, H-6<sub>A</sub>), 1.23 (d, 3H,  $J_{5,6} = 5.9$  Hz, H-6<sub>C</sub>), 0.90 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR: δ 170.9, 170.7, 169.6, 166.1, 162.1 (C=O), 138.3-118.1 (Ph, All), 101.5 (C-1<sub>D</sub>), 101.4 (C-1<sub>B</sub>), 101.1 (C-1<sub>A</sub>), 98.5 (C-1<sub>E</sub>), 96.4 (C-1<sub>C</sub>), 92.6 (CCl<sub>3</sub>), 82.1 (C-3<sub>E</sub>), 81.7 (C-3<sub>C</sub>), 81.6 (C-2<sub>E</sub>), 80.4 (C-4<sub>B</sub>), 80.1 (C-3<sub>A</sub>), 79.1 (bs, C-4<sub>C</sub>), 78.5 (C-3<sub>B</sub>), 77.9 (C-4<sub>A</sub>), 77.6 (C-4<sub>E</sub>), 76.4

(C-2<sub>A</sub>), 76.1, 75.8, 75.4, 74.7, 74.3, 74.2, 73.2, 70.4 (8C, CH<sub>2</sub>Ph), 74.9 (C-2<sub>B</sub>), 72.9 (C-3<sub>D</sub>), 72.7 (C-2<sub>C</sub>), 72.5 (C-5<sub>D</sub>), 71.9 (C-5<sub>E</sub>), 68.4 (C-6<sub>E</sub>), 68.8 (All), 68.9, 68.7, 68.5, 67.7 (4C, C-4<sub>D</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>), 62.1 (C-6<sub>D</sub>), 56.2 (C-2<sub>D</sub>), 20.9, 20.7 (3C, OAc), 19.0 (C-6<sub>A</sub>), 18.5 (C-6<sub>C</sub>), 18.2 (C-6<sub>B</sub>). FAB-MS of C<sub>104</sub>H<sub>114</sub>Cl<sub>3</sub>NO<sub>27</sub> (M, 1916.4) *m/z* 1938.9 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>104</sub>H<sub>114</sub>Cl<sub>3</sub>NO<sub>27</sub>: C, 65.18; H, 6.00; N, 0.73%. Found: C, 64.95; H, 6.17; N, 0.76%.

(2,3,4-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**346**). Compound **304** (3.5 g, 1.8 mmol) was dissolved in anhydrous THF (35 mL). The solution was degassed and placed under Argon. 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (81 mg) was added, and the solution was degassed again. The catalyst was activated by passing over a stream of hydrogen until the solution has turned yellow. The reaction mixture was degassed again and stirred under an Argon atmosphere, then concentrated to dryness. The residue was dissolved in acetone (15 mL), then water (3 mL), mercuric chloride (490 mg) and mercuric oxide (420 mg) were added successively. The mixture, protected from light, was stirred at rt for 2 h and acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aq KI, water and brine, dried and concentrated. The residue was eluted from a column of silica gel with 2:1 petroleum ether-EtOAc to give the corresponding hemiacetal **345**. Trichloroacetonitrile (6.5 mL) and DBU (97 μL) were added to a solution of the residue in anhydrous DCM (33 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 5:2 cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give **346** (2.48 g, 66 %) as a colourless foam: [α]<sub>D</sub> +4° (c 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR: δ 8.71 (s, 1H, NH), 8.00-7.00 (m, 45H, Ph), 6.80 (d, 1H, *J*<sub>2,NH</sub> = 8.6 Hz, NH<sub>D</sub>), 6.37 (d, 1H, *J*<sub>1,2</sub> = 2.7 Hz, H-1<sub>C</sub>), 5.59 (dd, 1H, *J*<sub>2,3</sub> = 2.9 Hz, H-2<sub>C</sub>), 5.10 (bs, 1H, H-1<sub>A</sub>), 5.05 (pt, 1H, *J*<sub>2,3</sub> = 9.8 Hz, H-3<sub>D</sub>), 5.02-4.96 (m, 4H, H-1<sub>E</sub>, 1<sub>B</sub>, 4<sub>D</sub>, CH<sub>2</sub>Ph), 5.00-4.42 (m, 17H, 15 CH<sub>2</sub>Ph, H-1<sub>D</sub>, 3<sub>C</sub>), 4.14 (bs, 1H, H-2<sub>A</sub>), 4.05-3.68 (m, 14H, H-3<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 4<sub>C</sub>, 5<sub>C</sub>, 2<sub>B</sub>, 3<sub>B</sub>, 3<sub>A</sub>, 5<sub>A</sub>, 2<sub>D</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>), 3.61 (dq, 1H, *J*<sub>5,6</sub> = 6.2, *J*<sub>4,5</sub> = 9.3 Hz, H-5<sub>B</sub>), 3.51-3.41 (m, 3H, H-2<sub>E</sub>, 4<sub>A</sub>, 5<sub>D</sub>), 3.30 (pt, 1H, *J*<sub>3,4</sub> = *J*<sub>4,5</sub> = 9.4 Hz, H-4<sub>B</sub>), 2.03, 2.02, 1.80 (3s, 9H, OAc), 1.39, 1.32 (2d, 6H, H-6<sub>A</sub>, 6<sub>C</sub>), 1.00 (bd, 3H, H-6<sub>B</sub>). <sup>13</sup>C NMR: δ 169.7, 169.5, 168.3, 164.5, 160.9 (C=O, C=N), 137.5-126.2 (Ph), 101.6 (C-1<sub>D</sub>), 101.3 (2C, C-1<sub>A</sub>, 1<sub>B</sub>), 98.7 (C-1<sub>E</sub>), 94.8 (C-1<sub>C</sub>), 91.3 (CCl<sub>3</sub>), 82.1, 81.5, 80.4, 80.1, 78.4, 77.9, 77.6, 76.5 (10C, C-2<sub>A</sub>, 2<sub>E</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>E</sub>), 76.0, 75.9, 75.5, 74.9, 74.3, 73.3 (8C, CH<sub>2</sub>Ph), 72.9, 72.6, 71.9, 70.9, 70.6, 69.1, 68.8, 68.5 (9C, C-2<sub>B</sub>, 2<sub>C</sub>, 3<sub>D</sub>, 4<sub>D</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>D</sub>, 5<sub>E</sub>), 68.3 (C-6<sub>E</sub>), 62.1 (C-6<sub>D</sub>), 56.2 (C-2<sub>D</sub>), 21.0, 20.9, 20.8 (3C, OAc), 19.1, 18.3, 18.1 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). Anal. Calcd for C<sub>103</sub>H<sub>110</sub>Cl<sub>6</sub>N<sub>2</sub>O<sub>27</sub>: C, 61.22; H, 5.49; N, 1.39%. Found: C, 61.24; H, 5.50; N, 1.21%.

**Methyl (2-Deoxy-4,6-*O*-isopropylidene-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosiden (348).** The pentasaccharide **302** (578 mg, 0.321 mmol) was dissolved in MeOH (10 mL). MeONa was added until pH reach 10. The mixture was stirred for 25 min then treated by IR 120 ( $H^+$ ) until neutral pH. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with 9:1 DCM-MeOH to give the expected triol **347** (505 mg, 89 %). To a mixture of **347** (505 mg, 0.286 mmol) in dry DMF (2 mL) was added 2-methoxypropene (60  $\mu$ L, 2.5 eq) and CSA (14 mg, cat). The mixture was stirred 1 h and  $Et_3N$  (200  $\mu$ L) was added. After evaporation, the residue was eluted from a column of silica gel with 5:2 cyclohexane-EtOAc with 0.3% of  $Et_3N$  to give **348** (420 mg, 81 %) as a colorless foam:  $^1H$  NMR:  $\delta$  8.00-7.00 (m, 45H, Ph), 7.17 (d, 1H,  $NH_D$ ), 5.39 (dd, 1H,  $J_{1,2} = 1.2$ ,  $J_{2,3} = 3.0$  Hz, H-2 $_C$ ), 5.13 (d, 1H,  $J_{1,2} = 1.1$  Hz, H-1 $_A$ ), 5.01 (d, 1H,  $J_{1,2} = 3.2$  Hz, H-1 $_E$ ), 4.99 (d, 1H,  $J_{1,2} = 1.7$  Hz, H-1 $_B$ ), 4.80 (d, 1H, H-1 $_C$ ), 4.70 (d, 1H, H-1 $_D$ ), 4.90-4.35 (m, 16H,  $CH_2Ph$ ), 4.40 (m, 1H, H-2 $_B$ ), 4.10 (dd, 1H, H-2 $_A$ ), 4.05 (dd, 1H, H-3 $_C$ ), 4.00-3.00 (m, 20H, H-4 $_C$ , 5 $_C$ , 3 $_B$ , 4 $_B$ , 5 $_B$ , 3 $_A$ , 4 $_A$ , 5 $_A$ , 2 $_D$ , 3 $_D$ , 4 $_D$ , 5 $_D$ , 6 $a_D$ , 6 $b_D$ , 2 $_E$ , 3 $_E$ , 4 $_E$ , 5 $_E$ , 6 $a_E$ , 6 $b_E$ ), 3.40 (s, 3H,  $OCH_3$ ), 1.40-1.00 (m, 15H,  $C(CH_3)_2$ , H-6 $_A$ , 6 $_B$ , 6 $_C$ );  $^{13}C$  NMR partial:  $\delta$  166.2, 164.4 ( $C=O$ ), 137.5-126.5 (Ph), 101.8 (C-1 $_D$ ), 101.4 (C-1 $_B$ ), 101.2 (C-1 $_A$ ), 100.2 ( $C(CH_3)_2$ ), 98.4 (C-1 $_E$ ), 98.2 (C-1 $_C$ ), 92.4 ( $CCl_3$ ), 68.5 (C-6 $_E$ ), 61.8 (C-6 $_D$ ), 60.1 (C-2 $_D$ ), 55.5 ( $OCH_3$ ), 29.3, 19.4 ( $C(CH_3)_2$ ), 19.1, 18.6, 18.2 (C-6 $_A$ , 6 $_B$ , 6 $_C$ ). FAB-MS of  $C_{99}H_{110}Cl_3N_1O_{24}$  (M, 1804.1),  $m/z$  1827.0 [ $M+Na$ ] $^+$ . Anal. Calcd for  $C_{99}H_{110}Cl_3N_1O_{24}$ : C, 65.90; H, 6.15; N, 0.78%. Found: C, 65.89; H, 6.29; N, 0.68%.

**Methyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-(2-deoxy-2-trichloroacetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (350).** A mixture of **346** (154 mg, 76  $\mu$ mol) and **348** (92 mg, 51  $\mu$ mol), 4 $\text{\AA}$  molecular sieves and dry 1,2-DCE (3 mL), was stirred for 1 h, then cooled to  $-35^\circ C$ . Triflic acid (6  $\mu$ L) was added. The stirred mixture was allowed to reach  $10^\circ C$  in 2.5 h.  $Et_3N$  (25  $\mu$ L) was added and the mixture was filtered. After evaporation, the residue was eluted from a column of silica gel with 2:1 cyclohexane-EtOAc and 0.5 % of  $Et_3N$  to give **349** (186 mg) as a contaminated material. To a solution of the isolated contaminated **349** (186 mg) in DCM (3 mL) was added dropwise, at  $0^\circ C$ , a solution of TFA (0.5 mL) and water (0.5 mL). The mixture was stirred for 3 h, then concentrated by co-evaporation with water then toluene. The residue was eluted from a column of silica gel with 2:1 to 1:1 petroleum ether-EtOAc to give **350** (134 mg, 72 %, 2

steps) as a white solid:  $[\alpha]_D +6^\circ$  (*c* 1.0,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR:  $\delta$  8.05-7.10 (m, 90H, Ph), 6.86-6.82 (2d, 2H,  $J_{2,\text{NH}} = 8.0$ ,  $J_{2,\text{NH}} = 8.5$  Hz,  $\text{NH}_D$ ,  $\text{NH}_D$ ), 5.35-5.19 (m, 2H, H-2<sub>C</sub>, 2<sub>C'</sub>), 5.20, 5.08 (2s, 2H, H-1<sub>A</sub>, 1<sub>A'</sub>), 5.05 (dd, 1H, H-3<sub>D'</sub>), 4.99-4.80 (m, 9H, H-1<sub>B</sub>, 1<sub>B'</sub>, 1<sub>C</sub>, 1<sub>C'</sub>, 1<sub>D</sub>, 1<sub>D'</sub>, 1<sub>E</sub>, 1<sub>E'</sub>, 4<sub>D'</sub>), 4.80-4.30 (m, 32H,  $\text{OCH}_2\text{Ph}$ ), 4.10-3.15 (m, 44H, H-2<sub>A</sub>, 2<sub>A'</sub>, 2<sub>B</sub>, 2<sub>B'</sub>, 2<sub>D</sub>, 2<sub>D'</sub>, 2<sub>E</sub>, 2<sub>E'</sub>, 3<sub>A</sub>, 3<sub>A'</sub>, 3<sub>B</sub>, 3<sub>B'</sub>, 3<sub>C</sub>, 3<sub>C'</sub>, 3<sub>D</sub>, 3<sub>E</sub>, 3<sub>E'</sub>, 4<sub>A</sub>, 4<sub>A'</sub>, 4<sub>B</sub>, 4<sub>B'</sub>, 4<sub>C</sub>, 4<sub>C'</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 4<sub>E'</sub>, 5<sub>A</sub>, 5<sub>A'</sub>, 5<sub>B</sub>, 5<sub>B'</sub>, 5<sub>C</sub>, 5<sub>C'</sub>, 5<sub>D</sub>, 5<sub>D'</sub>, 5<sub>E</sub>, 5<sub>E'</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>, 6<sub>aD'</sub>, 6<sub>bD'</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>, 6<sub>aE'</sub>, 6<sub>bE'</sub>), 3.42 (3H, s, OMe), 2.08, 2.04, 2.02 (9H, 3s, OAc), 1.40-0.96 (18H, m, H-6<sub>A</sub>, 6<sub>A'</sub>, 6<sub>B</sub>, 6<sub>B'</sub>, 6<sub>C</sub>, 6<sub>C'</sub>);  $^{13}\text{C}$  NMR:  $\delta$  171.5, 170.9, 170.8, 169.6, 166.2, 162.4, 162.1 (C=O), 139.5-127.2 (Ph), 101.9, 101.6, 101.5, 101.3, 99.2, 98.8, 98.2 (10C, C-1<sub>A</sub>, 1<sub>A'</sub>, 1<sub>B</sub>, 1<sub>B'</sub>, 1<sub>C</sub>, 1<sub>C'</sub>, 1<sub>D</sub>, 1<sub>D'</sub>, 1<sub>E</sub>, 1<sub>E'</sub>), 92.7, 92.6 (2C,  $\text{CCl}_3$ ), 82.1, 81.8, 81.7, 80.5, 80.3, 80.1, 79.3, 77.9, 77.8, 73.0, 72.6, 72.5, 72.0, 69.4, 69.0, 68.9, 67.4 (39C, C-2<sub>A</sub>, 2<sub>A'</sub>, 2<sub>B</sub>, 2<sub>B'</sub>, 2<sub>C</sub>, 2<sub>C'</sub>, 2<sub>E</sub>, 2<sub>E'</sub>, 3<sub>A</sub>, 3<sub>A'</sub>, 3<sub>B</sub>, 3<sub>B'</sub>, 3<sub>C</sub>, 3<sub>C'</sub>, 3<sub>D</sub>, 3<sub>D'</sub>, 3<sub>E</sub>, 3<sub>E'</sub>, 4<sub>A</sub>, 4<sub>A'</sub>, 4<sub>B</sub>, 4<sub>B'</sub>, 4<sub>C</sub>, 4<sub>C'</sub>, 4<sub>D</sub>, 4<sub>D'</sub>, 4<sub>E</sub>, 4<sub>E'</sub>, 5<sub>A</sub>, 5<sub>A'</sub>, 5<sub>B</sub>, 5<sub>B'</sub>, 5<sub>C</sub>, 5<sub>C'</sub>, 5<sub>D</sub>, 5<sub>D'</sub>, 5<sub>E</sub>, 5<sub>E'</sub>, 6<sub>D'</sub>), 76.0, 75.9, 74.8, 74.3, 73.6, 73.2, 68.6 ( $\text{CH}_2\text{Ph}$ ), 62.3, 62.2, 60.7 (3C, C-6<sub>D</sub>, 6<sub>E</sub>, 6<sub>E'</sub>), 55.5, 56.2 (3C, C-2<sub>D</sub>, 2<sub>D'</sub>,  $\text{OCH}_3$ ), 21.0, 20.9, 20.8 (OAc), 19.0, 18.7, 18.6, 18.2, 17.9 (6C, C-6<sub>A</sub>, 6<sub>A'</sub>, 6<sub>B</sub>, 6<sub>B'</sub>, 6<sub>C</sub>, 6<sub>C'</sub>). FAB-MS for  $\text{C}_{197}\text{H}_{214}\text{Cl}_6\text{N}_2\text{O}_{50}$  (M, 3622.5) *m/z* 3645.3  $[\text{M}+\text{Na}]^+$ . Anal. Calcd for  $\text{C}_{197}\text{H}_{214}\text{Cl}_6\text{N}_2\text{O}_{50}$ : C, 65.32; H, 5.95; N, 0.77%. Found: C, 65.20; H, 6.03; N, 0.78%.

**Methyl (2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-( $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-( $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-( $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-( $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-( $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranoside (301).** A solution of 350 (183 mg, 50  $\mu\text{mol}$ ), in EtOH (3 mL), EtOAc (0.3 mL), 1 M HCl (100  $\mu\text{L}$ ) was hydrogenated in the presence of Pd/C (250 mg) for 72 h at rt. The mixture was filtered and concentrated. A solution of the residue in MeOH (4 mL) and Et<sub>3</sub>N (200  $\mu\text{L}$ ) was hydrogenated in the presence of Pd/C (200 mg) for 24 h at rt. The mixture was filtered and concentrated. A solution of the residue (50 mg, 25  $\mu\text{mol}$ ) in MeOH (3 mL) and DCM (0.5 mL) was treated by MeONa until pH reached 10. The mixture was stirred overnight at 55°C. After cooling at rt, IR 120 ( $\text{H}^+$ ) was added until neutral pH, and the solution was filtered and concentrated, then was eluted from a column of C-18 with water/ $\text{CH}_3\text{CN}$  and freeze-dried to afford amorphous 301 (30 mg, 37 %):  $[\alpha]_D -1^\circ$  (*c* 1.0, water);  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ):  $\delta$  5.13 (2d, 2H,  $J_{1,2} = 3.5$  Hz, H-1<sub>E</sub>, 1<sub>E'</sub>), 5.05, 4.95, 4.75 (m, 5H, H-1<sub>A</sub>, 1<sub>B</sub>, 1<sub>A'</sub>, 1<sub>B'</sub>, 1<sub>C'</sub>), 4.64-4.62 (2d, 2H,  $J_{1,2} = 7.0$ ,  $J_{1,2} = 8.0$  Hz, H-1<sub>D</sub>, 1<sub>D'</sub>), 4.58 (d, 1H,  $J_{1,2} = 2.2$  Hz, H-1<sub>C</sub>), 4.10-3.20 (m, 51H, H-2<sub>A</sub>, 2<sub>A'</sub>, 2<sub>B</sub>, 2<sub>B'</sub>, 2<sub>C</sub>, 2<sub>C'</sub>, 2<sub>D</sub>, 2<sub>D'</sub>, 2<sub>E</sub>, 2<sub>E'</sub>, 3<sub>A</sub>, 3<sub>A'</sub>, 3<sub>B</sub>, 3<sub>B'</sub>, 3<sub>C</sub>, 3<sub>C'</sub>, 3<sub>D</sub>, 3<sub>D'</sub>, 3<sub>E</sub>, 3<sub>E'</sub>, 4<sub>A</sub>, 4<sub>A'</sub>, 4<sub>B</sub>, 4<sub>B'</sub>, 4<sub>C</sub>, 4<sub>C'</sub>, 4<sub>D</sub>, 4<sub>D'</sub>, 4<sub>E</sub>, 4<sub>E'</sub>, 5<sub>A</sub>, 5<sub>A'</sub>, 5<sub>B</sub>, 5<sub>B'</sub>, 5<sub>C</sub>, 5<sub>C'</sub>, 5<sub>D</sub>, 5<sub>D'</sub>, 5<sub>E</sub>, 5<sub>E'</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>, 6<sub>aD'</sub>, 6<sub>bD'</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>, 6<sub>aE'</sub>, 6<sub>bE'</sub>,  $\text{OCH}_3$ ), 1.99, 1.97 (2s, 6H, 2 NHAc), 1.33-1.15 (6d, 18H,  $J_{5,6} = 6.3$  Hz, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ ):  $\delta$  175.2, 174.7 (C=O), 103.1 (2C, C-1<sub>D'</sub>, 1<sub>D</sub>), 102.6, 101.7, 101.3, 100.8 (6C, C-1<sub>A</sub>, 1<sub>B</sub>, 1<sub>C</sub>, 1<sub>A'</sub>, 1<sub>B'</sub>, 1<sub>C'</sub>), 98.0 (2C, C-1<sub>E</sub>, 1<sub>E'</sub>), 81.6, 79.7, 79.6, 79.1, 76.2, 76.1, 73.9, 73.0,

72.7, 72.6, 72.5, 72.2, 72.1, 71.6, 70.1, 70.0, 69.7, 69.0, 68.5 (38C, C-2<sub>A</sub>, 2<sub>A'</sub>, 2<sub>B</sub>, 2<sub>B'</sub>, 2<sub>C</sub>, 2<sub>E</sub>, 2<sub>E'</sub>, 3<sub>A</sub>, 3<sub>A'</sub>, 3<sub>B</sub>, 3<sub>B'</sub>, 3<sub>C</sub>, 3<sub>C'</sub>, 3<sub>D</sub>, 3<sub>D'</sub>, 3<sub>E</sub>, 3<sub>E'</sub>, 4<sub>A</sub>, 4<sub>A'</sub>, 4<sub>B</sub>, 4<sub>B'</sub>, 4<sub>C</sub>, 4<sub>C'</sub>, 4<sub>D</sub>, 4<sub>D'</sub>, 4<sub>E</sub>, 4<sub>E'</sub>, 5<sub>A</sub>, 5<sub>A'</sub>, 5<sub>B</sub>, 5<sub>B'</sub>, 5<sub>C</sub>, 5<sub>C'</sub>, 5<sub>D</sub>, 5<sub>D'</sub>, 5<sub>E</sub>, 5<sub>E'</sub>), 60.9 (4C, C-6<sub>E</sub>, 6<sub>E'</sub>, 6<sub>D</sub>, 6<sub>D'</sub>), 56.2, 56.0, 55.3 (3C-2<sub>D</sub>, 2<sub>D'</sub>, OCH<sub>3</sub>), 22.7, 22.6 (2C, NHAc), 18.3, 18.1, 17.2, 17.1, 17.0, 16.9 (6C, C-6<sub>A</sub>, 6<sub>A'</sub>, 6<sub>B</sub>, 6<sub>B'</sub>, 6<sub>C</sub>, 6<sub>C'</sub>). HRMS (MALDI) calcd for [C<sub>65</sub>H<sub>110</sub>N<sub>2</sub>O<sub>45</sub>+Na]<sup>+</sup>: 1661.6278. Found: 1661.6277.

**D- Synthesis of the 2-amionoethyl glycoside of a hapt representative of the O-specific polysaccharide of *Shigella flexneri* serotype 2a and a corresponding PADRE-conjugate**

(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**406**). 1,5-Cyclooctadiene bis(methyldiphenylphosphine)iridium hexafluorophosphate (25 mg, 29 μmol) was dissolved in THF (5 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **407** (1.0 g, 0.5 mmol) in THF (10 mL) was degassed and added. The mixture was stirred at rt overnight then concentrated to dryness. The residue was dissolved in acetone (5 mL), then water (1 mL), mercuric chloride (140 mg) and mercuric oxide (120 mg) were added successively. The mixture protected from light was stirred at rt for 2 h and acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aq KI, water and satd aq NaCl, dried and concentrated. The residue was eluted from a column of silica gel with 2:1 petroleum ether-EtOAc to give the corresponding hemiacetal **408**. Trichloroacetonitrile (2.5 mL) and DBU (37 μL) were added to a solution of the crude **408** in anhydrous DCM (12.5 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 5:4 cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give **406** as a white foam (0.9 g, 85 %); [α]<sub>D</sub> +10° (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR: δ 8.70 (s, 1H, C=NH), 8.00-7.00 (m, 45H, Ph), 6.36 (d, 1H, *J*<sub>1,2</sub> = 2.6 Hz, H-1<sub>C</sub>), 5.59 (m, 2H, N-H<sub>D</sub>, H-2<sub>C</sub>), 5.13 (d, 1H, *J*<sub>1,2</sub> = 1.0 Hz, H-1<sub>A</sub>), 5.01-4.98 (m, 2H, H-1<sub>E</sub>, 1<sub>B</sub>), 4.92 (dd, 1H, H-3<sub>D</sub>), 4.90 (dd, 1H, H-4<sub>D</sub>), 4.68 (d, 1H, H-1<sub>D</sub>), 5.00-4.02 (m, 19H, 8 CH<sub>2</sub>Ph, H-3<sub>C</sub>, 2<sub>A</sub>, 2<sub>B</sub>), 4.01 (dd, 1H, H-2<sub>E</sub>), 4.00-3.20 (m, 16H, H-3<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 4<sub>C</sub>, 5<sub>C</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 5<sub>B</sub>, 3<sub>A</sub>, 4<sub>A</sub>, 5<sub>A</sub>, 5<sub>D</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>), 2.02, 2.00, 1.75, 1.65 (4s, 12H, C=OCH<sub>3</sub>), 1.40, 1.32 and 1.00 (3d, 9H, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR (partial): δ 170.2, 169.9, 169.3, 168.7, 164.9 (6C, C=O, C=N), 103.2 (C-1<sub>D</sub>), 101.4 (2C, C-1<sub>A</sub>, 1<sub>B</sub>), 99.0 (C-1<sub>E</sub>), 94.8 (C-1<sub>C</sub>), 21.1, 20.9, 20.8 (3C, CH<sub>3</sub>C=O), 19.1, 18.2 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). Anal. Calcd for C<sub>103</sub>H<sub>113</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>27</sub>: C, 64.52; H, 5.94; N, 1.46%. Found: C, 64.47; H, 5.99; N, 1.45%.

2-Azidoethyl                      (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-  
glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-  
benzyl-α-*L*-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl-  
(1→4)]-(2-*O*-benzoyl-α-*L*-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-

5 isopropylidene-β-*D*-glucopyranoside (409). A mixture of alcohol 405 (110 mg, 330 μmol), trichloroacetimidate 406 (720 mg, 376 μmol) and 4 Å molecular sieves in anhydrous 1,2-DCE (6 mL) was stirred for 1 h under dry argon. After cooling at 0°C, TfOH (16 μL, 180 μmol) was added dropwise and the mixture was stirred at 80°C for 2.5 h. Triethylamine (60 μL) was added and the mixture was filtered, and concentrated. The  
10 residue was eluted from a column of silica gel with 3:4 cyclohexane-EtOAc and Et<sub>3</sub>N (0.2 %) to give 409 as a colourless oil (540 mg, 78 %); [α]<sub>D</sub> +6.5° (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR: δ 8.00-7.00 (m, 45H, Ph), 5.95 (d, 1H, *J*<sub>2,NH</sub> = 7.1 Hz, NH<sub>D</sub>), 5.51 (d, 1H, *J*<sub>2,NH</sub> = 8.1 Hz, NH<sub>D</sub>), 5.20 (dd, 1H, *J*<sub>1,2</sub> = 1.7, *J*<sub>2,3</sub> = 3.0 Hz, H-2<sub>C</sub>), 5.08 (d, 1H, *J*<sub>1,2</sub> = 1.0 Hz, H-1<sub>A</sub>), 5.05 (d, 1H, *J*<sub>1,2</sub> = 8.3 Hz, H-1<sub>D</sub>), 4.93 (d, 1H, *J*<sub>1,2</sub> = 3.1 Hz, H-1<sub>E</sub>), 4.87 (d, 1H, *J*<sub>1,2</sub> = 1.0 Hz, H-1<sub>B</sub>), 4.82 (d, 1H, *J*<sub>1,2</sub> = 1.7 Hz, H-1<sub>C</sub>), 4.80 (dd, 1H, *J*<sub>3,4</sub> = *J*<sub>4,5</sub> = 10.0 Hz, H-4<sub>D</sub>), 4.76 (dd, 1H, *J*<sub>2,3</sub> = 9.5 Hz, H-3<sub>D</sub>), 4.75-4.30 (m, 16H, CH<sub>2</sub>Ph), 4.57 (d, 1H, *J*<sub>1,2</sub> = 7.8 Hz, H-1<sub>D</sub>), 4.35 (dd, 1H, H-2<sub>B</sub>), 4.30 (dd, 1H, *J*<sub>2,3</sub> = 10.0, *J*<sub>3,4</sub> = 9.6 Hz, H-3<sub>D</sub>), 4.02 (dd, 1H, *J*<sub>2,3</sub> = 2.0 Hz, H-2<sub>A</sub>), 4.00-3.60 (m, 16H, H-6<sub>A</sub>, 6<sub>B</sub>, 3<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>, 6<sub>A</sub>, 6<sub>B</sub>, 3<sub>C</sub>, 4<sub>C</sub>, 5<sub>C</sub>, 3<sub>B</sub>, 3<sub>A</sub>, 5<sub>A</sub>, 2<sub>D</sub>, 6<sub>A</sub>, 6<sub>B</sub>), 3.48 (m, 1H, *J*<sub>4,5</sub> = 9.5 Hz, H-5<sub>B</sub>), 3.46 (dd, 1H, H-4<sub>D</sub>), 3.40 (m, 1H, H-5<sub>D</sub>), 3.36 (dd, 1H, H-2<sub>E</sub>), 3.35, 3.19 (m, 4H, OCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.30 (dd, 1H, H-4<sub>A</sub>), 3.19 (dd, 1H, *J*<sub>3,4</sub> = 9.5 Hz, H-4<sub>B</sub>), 3.17 (m, 1H, H-5<sub>D</sub>), 3.02 (m, 1H, H-2<sub>D</sub>), 1.90-1.60 (6s, 18H, CH<sub>3</sub>C=O), 1.33, 1.26 (2s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 1.27 (d, 1H, *J*<sub>5,6</sub> = 6.2 Hz, H-6<sub>A</sub>), 1.18 (d, 3H, *J*<sub>5,6</sub> = 6.1 Hz, H-6<sub>C</sub>), 0.90 (d, 3H, *J*<sub>5,6</sub> = 6.1 Hz, H-6<sub>B</sub>). <sup>13</sup>C NMR: δ 172.1, 171.1, 170.8, 170.1, 169.6, 166.2 (6C, C=O), 139.2-127.1 (Ph), 103.1<sub>A</sub> (C-1<sub>D</sub>), 101.6 (C-1<sub>B</sub>), 101.0 (C-1<sub>A</sub>), 100.0 (C-1<sub>D</sub>), 98.1 (C-1<sub>E</sub>), 97.8 (C-1<sub>C</sub>), 82.0 (C-2<sub>E</sub>), 81.7, 81.5, 80.2, 78.6, 78.4, 77.9, 77.9 (8C, C-3<sub>E</sub>, 4<sub>E</sub>, 3<sub>C</sub>, 4<sub>C</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 3<sub>A</sub>, 4<sub>A</sub>), 77.8 (C-2<sub>A</sub>), 76.0, 74.6 (2C, C-3<sub>D</sub>, 3<sub>D</sub>), 74.0 (C-2<sub>B</sub>), 73.4 (C-4<sub>D</sub>), 73.3 (C-2<sub>C</sub>), 72.2, 71.9 (2C, C-5<sub>D</sub>, 5<sub>D</sub>), 68.9, 68.8, 67.7 (3C, C-5<sub>A</sub>, 5<sub>B</sub>, 5<sub>E</sub>), 68.6 (C-4<sub>D</sub>), 68.5 (C-6<sub>E</sub>), 67.5 (C-5<sub>C</sub>), 62.6, 62.2 (2C, C-6<sub>D</sub>, 6<sub>D</sub>), 59.7 (C-2<sub>D</sub>), 54.6 (C-2<sub>D</sub>), 51.0 (CH<sub>2</sub>N<sub>3</sub>), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 23.9, 23.5, 21.1, 20.9, 20.7 (5C, C=OCH<sub>3</sub>), 19.6 (C(CH<sub>3</sub>)<sub>2</sub>), 18.9 (C-6<sub>C</sub>), 18.4 (C-6<sub>A</sub>), 18.2 (C-6<sub>B</sub>). FABMS of C<sub>114</sub>H<sub>133</sub>N<sub>5</sub>O<sub>32</sub> (M, 2085.3) *m/z* 2107.9 [M+Na]<sup>+</sup>

2-Azidoethyl                      (2,3,4-tri-*O*-acetyl-2-deoxy-2-acetamido-β-*D*-  
glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-*L*-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-  
benzyl-α-*L*-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl-  
(1→4)]-(2-*O*-benzoyl-α-*L*-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-*D*-  
35 glucopyranoside (410). To a solution of 409 (503 mg, 241 μmol) in AcOH (6 mL) was added water (1.5 mL) dropwise at rt. The mixture was stirred for 1 h at 60°C then concentrated by successive co-evaporation with water and toluene. The residue was eluted

from a column of silica gel with 1:4 Cyclohexane-EtOAc to give **410** as a white foam (463 mg, 94 %);  $[\alpha]_D^{+9}$  (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  8.00-7.00 (m, 45H, Ph), 5.70 (d, 1H, NH<sub>D</sub>), 5.46 (d, 1H,  $J_{2,NH}$  = 8.0 Hz, NH<sub>D</sub>), 5.25 (dd, 1H, H-2<sub>C</sub>), 5.05 (d, 1H,  $J_{1,2}$  = 8.4 Hz, H-1<sub>D</sub>), 5.00 (d, 1H,  $J_{1,2}$  = 1.0 Hz, H-1<sub>A</sub>), 4.86 (m, 3H, H-1<sub>C</sub>, 3<sub>D</sub>, 4<sub>D</sub>), 4.84 (m, 2H, H-1<sub>B</sub>, 1<sub>E</sub>), 4.56 (d, 1H, H-1<sub>D</sub>), 4.40 (dd, 1H, H-3<sub>E</sub>), 4.35 (dd, 1H, H-2<sub>B</sub>), 4.15 (dd, 1H, H-3<sub>D</sub>), 4.80-4.00 (m, 16H, CH<sub>2</sub>Ph), 4.03 (dd, 1H, H-2<sub>A</sub>), 4.00-3.00 (m, 26H, H-4<sub>D</sub>, 5<sub>D</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>, 2<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 3<sub>C</sub>, 4<sub>C</sub>, 5<sub>C</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 5<sub>B</sub>, 3<sub>A</sub>, 4<sub>A</sub>, 5<sub>A</sub>, 2<sub>D</sub>, 5<sub>D</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>, OCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 2.99 (m, 1H, H-2<sub>D</sub>), 1.85-1.60 (5s, 15H, CH<sub>3</sub>C=O), 1.25 and 0.85 (3d, 9H, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR (partial):  $\delta$  171.6, 171.4, 170.8, 170.1, 169.6 (C=O), 140.0-127.1 (Ph), 103.1 (C-1<sub>D</sub>), 101.2 (C-1<sub>A</sub>), 99.6 (2C, C-1<sub>E</sub>, 1<sub>B</sub>), 99.4 (C-1<sub>D</sub>), 99.0 (C-1<sub>C</sub>), 23.8, 23.5 (2C, NHAc), 21.1, 20.9, 20.8 (3C, OAc), 19.1, 18.5, 18.2 (C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). FABMS of C<sub>111</sub>H<sub>129</sub>N<sub>5</sub>O<sub>32</sub> (M, 2045.2), *m/z* 2067.9 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>111</sub>H<sub>129</sub>N<sub>5</sub>O<sub>32</sub>: C, 65.19; H, 6.36; N, 3.42%. Found: C, 65.12; H, 6.51; N, 3.41%.

**2-Aminoethyl (2-deoxy-2-acetamido- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-( $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-( $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-( $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (**402**).** A solution of **410** (207 mg, 101  $\mu$ mol) in MeOH (5 mL) was treated by MeONa until pH 9. The mixture was stirred 1 week at rt. IR 120 (H<sup>+</sup>) was added until neutral pH and the solution was filtered, and concentrated. The residue was eluted from a column of silica gel with 20:1 to 15:1 DCM-MeOH to give amorphous **411**. A solution of crude **411** in EtOH (2.2 mL), EtOAc (220  $\mu$ L), 1 M HCl (172  $\mu$ L, 2 eq) was hydrogenated in the presence of Pd/C (180 mg) for 72 h at rt. The mixture was filtered and concentrated. Elution of the residue from a column of C18 with water and freeze-drying of appropriate fractions resulted in amorphous **402** (81 mg, 77 %);  $[\alpha]_D^{-10}$  (*c* 1, water). <sup>1</sup>H NMR partial (D<sub>2</sub>O):  $\delta$  5.12 (d, 1H,  $J_{1,2}$  = 3.4 Hz, H-1<sub>E</sub>), 5.07 (d, 1H,  $J_{1,2}$  = 1.0 Hz, H-1<sub>Rha</sub>), 4.94 (d, 1H,  $J_{1,2}$  = 1.0 Hz, H-1<sub>Rha</sub>), 4.75 (d, 1H,  $J_{1,2}$  = 1.0 Hz, H-1<sub>Rha</sub>), 4.63 (d, 1H,  $J_{1,2}$  = 8.35 Hz, H-1<sub>GlcNac</sub>), 4.54 (d, 1H,  $J_{1,2}$  = 8.3 Hz, H-1<sub>GlcNac</sub>), 1.98 and 1.96 (2s, 6H, 2 CH<sub>3</sub>C=ONH), 1.28-1.20 (m, 9H, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR partial (D<sub>2</sub>O):  $\delta$  175.2, 174.8 (2C, C=O), 103.1 (C-1<sub>D</sub>), 101.6, 101.4 (3C, C-1<sub>A</sub>, 1<sub>B</sub>, 1<sub>C</sub>), 100.8 (C-1<sub>D</sub>), 97.9 (C-1<sub>E</sub>), 56.2, 55.4 (2C, C-2<sub>D</sub>, 2<sub>D</sub>), 22.7, 22.6 (2 NHAc), 18.2, 17.2, 17.0 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). HRMS (MALDI) Calcd for C<sub>42</sub>H<sub>73</sub>N<sub>3</sub>O<sub>28</sub>Na: 1090.4278. Found 1090.4286.

**(S-Acetylthiomethyl)carbonylaminoethyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (**412**).** A solution of **404** (3.4 mg, 11.4  $\mu$ mol) in CH<sub>3</sub>CN (50  $\mu$ L) was added to the aminoethyl hexasaccharide **402** (4.1 mg, 3.84  $\mu$ mol) in 0.1 M phosphate buffer (pH 7.4, 500  $\mu$ L). The mixture was stirred at rt for 1 h and purified by RP-HPLC to give **412** (2.7 mg, 59%). HPLC (230 nm): Rt 14.27 min (99.9% pure, Kromasil 5  $\mu$ m C18

100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01 M aq TFA at 1 mL/min flow rate). ES-MS for C<sub>46</sub>H<sub>77</sub>N<sub>3</sub>O<sub>30</sub>S (M, 1184.19) *m/z* 1184.08.

**PADRE (thiomethyl)carbonylaminoethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (401).** Compound 412 (4.9 mg, 4.12 μmol) was dissolved in water (350 μL) and added to a solution of 403 (9.1 mg, 5.2 μmol) in a mixture of water (750 μL), CH<sub>3</sub>CN (150 μL) and 0.5 M phosphate buffer (pH 5.6, 900 μL). 89 μL of a solution of hydroxylamine hydrochloride (139 mg/mL) in 0.5 M phosphate buffer (pH 5.6) was added and the mixture was stirred for 2 h. RP-HPLC purification gave the pure target 401 (6.3 mg, 53%). HPLC (230 nm): Rt 9.70 min (100% pure, Kromasil 5 μm C18 100 Å 4.6x250 mm analytical column, using a 20-50% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01 M aq TFA at 1 mL/min flow rate). ES-MS Calcd for C<sub>153</sub>H<sub>254</sub>N<sub>24</sub>O<sub>65</sub>S (M, 2901.34) *m/z* 2901.20.

**E- Preparation of chemically defined glycopeptides as potential synthetic conjugate vaccines against *Shigella flexneri* serotype 2a disease**

Solvent mixtures of appropriately adjusted polarity used for chromatography consisted of *A*, dichloromethane-methanol; *B*, cyclohexane-ethyl acetate, *C*, cyclohexane-acetone, *D*, toluene-ethyl acetate.

**2-Azidoethyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (507).** Camphorsulfonic acid (200 mg, 0.9 mmol) was added to a solution of triol 514 (1.31 g, 4.52 mmol) in a mixture of DMF (4 mL) and 2,2-dimethoxypropane (4 mL). After 3 h at rt, low boiling point solvents were evaporated under reduced pressure and more 2,2-dimethoxypropane (2 mL, 15.8 mmol) was added. The mixture was stirred for 2h at rt, Et<sub>3</sub>N was added, and the mixture was concentrated. The crude product was purified by column chromatography (solvent A, 19:1) to give 507 as a white solid (1.21 g, 81%), [α]<sub>D</sub> -89.8; <sup>1</sup>H NMR: δ 6.15 (d, 1H, *J* = 5.9 Hz, NH), 4.70 (d, 1H, *J*<sub>1,2</sub> = 8.3 Hz, H-1), 4.05 (m, 1H, OCH<sub>2</sub>), 3.97-3.89 (m, 2H, H-6a, 3), 3.79 (pt, 1H, *J*<sub>5,6b</sub> = *J*<sub>6a,6b</sub> = 10.5 Hz, H-6b), 3.70 (m, 1H, OCH<sub>2</sub>), 3.62-3.46 (m, 3H, H-2, 4, OCH<sub>2</sub>), 3.35-3.26 (m, 2H, H-5, CH<sub>2</sub>N<sub>3</sub>), 2.05 (s, 3H, Ac), 1.52 (s, 3H, C(CH<sub>3</sub>)<sub>2</sub>), 1.44 (s, 3H, C(CH<sub>3</sub>)<sub>2</sub>); <sup>13</sup>C NMR: δ 100.9 (C-1), 74.3 (C-4), 81.8 (C-3), 68.6 (OCH<sub>2</sub>), 67.3 (C-5), 62.0 (C-6), 58.7 (C-2), 50.7 (CH<sub>2</sub>N<sub>3</sub>), 29.0 (C(CH<sub>3</sub>)<sub>2</sub>), 23.6 (CH<sub>3</sub>CO), 19.1 (C(CH<sub>3</sub>)<sub>2</sub>). CIMS for C<sub>13</sub>H<sub>22</sub>N<sub>4</sub>O<sub>6</sub> (330) *m/z* 331 [M+H]<sup>+</sup>. Anal. Calcd. for C<sub>67</sub>H<sub>74</sub>N<sub>4</sub>O<sub>17</sub>·0.5H<sub>2</sub>O: C, 46.01; H, 6.83; N, 16.51%. Found C, 46.37; H, 6.69; N, 16.46%.

**2-Azidoethyl (2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (515) and 2-Azidoethyl (2,3,4,6-tetra-*O*-benzyl-α-**



**D-glucopyranosyl)-(1→4)-(2,3-di-O-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1→3)-2-**

**acetamido-2-deoxy- $\beta$ -D-glucopyranoside (516).** (a) The disaccharide donor **504** (1.425 g, 1.37 mmol) and the acceptor **507** (377 mg, 1.14 mmol) with 4Å-MS (2 g) were placed under argon and CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added. The mixture was stirred for 1 h at rt, then cooled to -40°C. A solution of BF<sub>3</sub>·OEt<sub>2</sub> (0.5 mL, 4.11 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added dropwise. The mixture was stirred at -40°C to -15°C over 3 h. Triethylamine (2.5 mL) was added and the mixture stirred for 20 min. The mixture was filtered through a pad of Celite, and the filtrate was concentrated. The mixture was purified by column chromatography (solvent B, 2:3) to give **515** (803 mg, 58%) as a colourless foam. Further elution (solvent A, 9:1) gave **516** (395 mg, 30%) as a colourless foam. Compound **516** had  $[\alpha]_D +91.5$  (c 0.18); <sup>1</sup>H NMR:  $\delta$  6.99-8.02 (m, 30H, Ph), 6.10 (d, 1H,  $J_{NH,2} = 6.9$  Hz, NH), 5.60 (dd, 1H,  $J_{2,3} = 3.4$ ,  $J_{3,4} = 9.1$  Hz, H-3<sub>C</sub>), 5.52 (dd, 1H, H-2<sub>C</sub>), 5.20 (d, 1H,  $J_{1,2} = 8.3$  Hz, H-1<sub>D</sub>), 5.00 (d, 1H,  $J_{1,2} = 1.9$  Hz, H-1<sub>C</sub>), 4.95 (d, 1H,  $J_{1,2} = 3.4$  Hz, H-1<sub>E</sub>), 4.89-4.63 (m, 5H, CH<sub>2</sub>Ph), 4.47 (dd, 1H,  $J_{2,3} = 8.3$ ,  $J_{3,4} = 10.3$  Hz, H-3<sub>D</sub>), 4.25 (d, 1H,  $J = 10.9$  Hz, CH<sub>2</sub>Ph), 4.19 (m, 2H, H-5<sub>C</sub>, CH<sub>2</sub>Ph), 4.06 (m, 1H, CH<sub>2</sub>O), 3.87 (m, 5H, H-3<sub>E</sub>, 4<sub>C</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>, CH<sub>2</sub>Ph), 3.74-3.58 (m, 4H, H-4<sub>E</sub>, 5<sub>D</sub>, 5<sub>E</sub>, CH<sub>2</sub>O), 3.50 (m, 3H, H-2<sub>E</sub>, 4<sub>D</sub>, CH<sub>2</sub>N<sub>3</sub>), 3.32 (d, 1H,  $J_{6a,6b} = 9.6$  Hz, H-6a<sub>E</sub>), 3.26 (m, 1H, CH<sub>2</sub>N<sub>3</sub>), 3.04 (d, 2H, H-2<sub>D</sub>, 6b<sub>E</sub>), 2.02 (s, 3H, CH<sub>3</sub>CO), 1.51 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>C</sub>); <sup>13</sup>C NMR:  $\delta$  171.5, 165.6, 165.2 (3C, C=O), 138.6-127.3 (Ph), 99.6 (C-1<sub>C</sub>), 99.5 (C-1<sub>E</sub>), 99.0 (C-1<sub>D</sub>), 83.4 (C-3<sub>D</sub>), 81.6 (C-3<sub>E</sub>), 80.1 (C-2<sub>E</sub>), 79.2 (C-4<sub>C</sub>), 77.2 (C-4<sub>E</sub>), 75.5 (CH<sub>2</sub>Ph), 75.1 (C-4<sub>D</sub>), 74.7, 74.0, 73.2 (3C, CH<sub>2</sub>Ph), 71.3 (C-5<sub>D</sub>\*), 70.9 (C-5<sub>E</sub>\*), 70.8 (C-3<sub>C</sub>), 70.4 (C-2<sub>C</sub>), 69.0 (C-5<sub>C</sub>), 68.8 (CH<sub>2</sub>O), 67.5 (C-6<sub>E</sub>), 62.6 (C-6<sub>D</sub>), 57.9 (C-2<sub>D</sub>), 50.5 (CH<sub>2</sub>N<sub>3</sub>), 23.4 (CH<sub>3</sub>CO), 18.2 (C-6<sub>C</sub>). FAB-MS for C<sub>64</sub>H<sub>70</sub>N<sub>4</sub>O<sub>17</sub> (M, 1166)  $m/z$  1185 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>64</sub>H<sub>70</sub>N<sub>4</sub>O<sub>17</sub>·H<sub>2</sub>O: C, 64.85; H, 6.12; N, 4.73%. Found: C, 64.71; H, 6.01; N, 4.83%.

(b) 4 Å Molecular sieves (560 mg) were added to a solution of donor **504** (565 mg, 0.54 mmol) and acceptor **507** (150 mg, 0.45 mmol) in DCM (3 mL) and the suspension was stirred for 15 min -40°C. Triflic acid (16  $\mu$ L) was added and the mixture was stirred for 3h at rt once the cooling bath had reached rt. Et<sub>3</sub>N was added and after 15 min, the mixture was filtered through a pad of Celite. Volatiles were evaporated and the residue was column chromatographed (solvent B, 9:1) to give **515** (475 mg, 87%).  $[\alpha]_D +87.7$  (c 0.32); <sup>1</sup>H NMR:  $\delta$  8.07-6.99 (m, 30H, Ph), 6.21 (d, 1H, NH), 5.58 (dd, 1H, H-3<sub>C</sub>), 5.44 (m, 1H, H-2<sub>C</sub>), 5.13 (d, 1H,  $J_{1,2} = 8.3$  Hz, H-1<sub>D</sub>), 5.02 (d, 1H,  $J_{1,2} = 3.4$  Hz, H-1<sub>E</sub>), 4.97 (d, 1H,  $J_{1,2} = 1.5$  Hz, H-1<sub>C</sub>), 4.64-4.90 (m, 5H, CH<sub>2</sub>Ph), 4.45 (t, 1H, H-3<sub>D</sub>), 4.27 (m, 3H, H-5<sub>C</sub>, CH<sub>2</sub>Ph), 4.05-3.79 (m, 7H, H-3<sub>E</sub>, 4<sub>C</sub>, 5<sub>D</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>, CH<sub>2</sub>O, CH<sub>2</sub>Ph), 3.60-3.76 (m, 4H, H-4<sub>D</sub>, 4<sub>E</sub>, 5<sub>E</sub>, CH<sub>2</sub>O), 3.37-3.51 (m, 3H, H-2<sub>E</sub>, 5<sub>D</sub>, CH<sub>2</sub>N<sub>3</sub>), 3.34-3.16 (m, 3H, H-2<sub>D</sub>, 6a<sub>E</sub>, CH<sub>2</sub>N<sub>3</sub>), 3.04 (d, 1H, H-6b<sub>E</sub>), 2.01 (s, 3H, CH<sub>3</sub>C=O), 1.43 (s, 6H, (CH<sub>3</sub>)<sub>2</sub>C), 1.36 (d, 3H, H-6<sub>C</sub>); <sup>13</sup>C NMR:  $\delta$  171.7, 165.6, 163.4 (C=O), 138.6-127.3 (Ph), 99.6 (C-1<sub>D</sub>), 99.1 (C-1<sub>E</sub>), 97.7 (C-1<sub>C</sub>), 91.9 ((CH<sub>3</sub>)<sub>2</sub>C), 81.4 (C-3<sub>E</sub>), 80.3 (C-2<sub>E</sub>), 79.4 (C-4<sub>C</sub>), 77.1 (C-4<sub>D</sub>), 76.0 (C-

3<sub>D</sub>), 75.3, 74.6, 73.9, 73.2 (4C, CH<sub>2</sub>Ph), 73.1 (C-4<sub>E</sub>), 71.2 (2C, C-2<sub>C</sub>, 3<sub>C</sub>), 71.1 (C-5<sub>E</sub>), 68.6 (CH<sub>2</sub>O), 67.5 (C-5<sub>C</sub>), 67.4 (C-6<sub>E</sub>), 67.1 (C-5<sub>D</sub>), 62.1 (C-6<sub>D</sub>), 59.0 (C-2<sub>D</sub>), 50.5 (CH<sub>2</sub>N<sub>3</sub>), 28.9 ((CH<sub>3</sub>)<sub>2</sub>C), 23.4 (CH<sub>3</sub>CO), 19.2 ((CH<sub>3</sub>)<sub>2</sub>C), 18.1 (C-6<sub>C</sub>). FAB-MS for C<sub>67</sub>H<sub>74</sub>N<sub>4</sub>O<sub>17</sub> (1206) *m/z* 1229 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>67</sub>H<sub>74</sub>N<sub>4</sub>O<sub>17</sub>: C, 60.41; H, 5.66; N, 4.82%.

5 Found: C, 60.36; H, 5.69; N, 4.78%.

**2-Azidoethyl (2,3,4,6-Tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (517).** An ice cold solution of 95% aq TFA (1.5 mL) in CH<sub>2</sub>Cl<sub>2</sub> (13.5 mL) was added to the trisaccharide 515 (730 mg, 0.60 mmol). The mixture was kept at 0°C for 15 min, then diluted with toluene and concentrated. Toluene was co-evaporated from the residue. The residue was dissolved in MeOH (20 mL), and a 1M solution of sodium methoxide in MeOH (1.5 mL) was added. The mixture was left to stand at rt for 3 h. The mixture was neutralised with Amberlite IR-120 (H<sup>+</sup>) resin and filtered. The filtrate was concentrated. The mixture was purified by column chromatography (solvent A, 9:1) to give 517 (548 mg, 94%) as a colourless foam. [ $\alpha$ ]<sub>D</sub> +9.7 (*c* 0.48, MeOH); <sup>1</sup>H NMR:  $\delta$  7.13-7.31 (m, 8H, Ph), 5.99 (d, 1H, J<sub>NH,2</sub> = 7.8 Hz, NH), 4.97-4.79 (m, 7H, H-1<sub>C</sub>, 1<sub>D</sub>, 1<sub>E</sub>, CH<sub>2</sub>Ph), 4.374-4.35 (m, 4H, CH<sub>2</sub>Ph), 4.10-3.91 (m, 7H, H-2<sub>C</sub>, 3<sub>D</sub>, 3<sub>E</sub>, 5<sub>C</sub>, 5<sub>E</sub>, 6a<sub>D</sub>, CH<sub>2</sub>O), 3.80 (m, 2H, H-3<sub>E</sub>, 6b<sub>D</sub>), 3.73 (m, 1H, CH<sub>2</sub>O), 3.40-3.63 (m, 8H, H-2<sub>E</sub>, 4<sub>C</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>D</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, CH<sub>2</sub>N<sub>3</sub>), 3.27 (m, 2H, H-2<sub>D</sub>, CH<sub>2</sub>N<sub>3</sub>), 1.99 (s, 3H, CH<sub>3</sub>CO), 1.41 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>C</sub>); <sup>13</sup>C NMR:  $\delta$  170.7 (C=O), 138.4-127.6 (Ph), 101.2 (C-1<sub>C</sub>), 99.7 (C-1<sub>E</sub>), 99.0 (C-1<sub>D</sub>), 84.7 (C-4<sub>C</sub>), 84.3 (C-3<sub>D</sub>), 81.5 (C-3<sub>E</sub>), 79.6 (C-2<sub>E</sub>), 77.6 (C-4<sub>D</sub>\*), 75.6 (CH<sub>2</sub>Ph), 75.3 (C-4<sub>E</sub>\*), 74.9, 73.5, 73.4 (3C, CH<sub>2</sub>Ph), 71.2 (C-5<sub>E</sub>), 70.8 (C-5<sub>C</sub>), 70.8 (C-5<sub>D</sub>), 69.4 (C-3<sub>C</sub>), 68.6 (C-6<sub>E</sub>), 68.4 (CH<sub>2</sub>O), 67.6 (C-2<sub>C</sub>), 62.6 (C-6<sub>D</sub>), 56.4 (C-2<sub>D</sub>), 50.5 (CH<sub>2</sub>N<sub>3</sub>), 23.5 (CH<sub>3</sub>CO), 17.6 (C-6<sub>C</sub>). FAB-MS for C<sub>50</sub>H<sub>62</sub>N<sub>4</sub>O<sub>15</sub> (958) *m/z* 981 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>50</sub>H<sub>62</sub>N<sub>4</sub>O<sub>15</sub>·H<sub>2</sub>O: C, 61.46; H, 6.60; N, 5.73%. Found: C, 61.41; H, 6.61; N, 5.97%.

**2-Aminoethyl  $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (518).** The trisaccharide 517 (368 mg, 0.38 mmol) was dissolved in a mixture of EtOH (10 mL) and EtOAc (1 mL). A 1N solution of aqueous HCl (0.77 mL) was added. The mixture was stirred under hydrogen in the presence of 10% Pd/C (400 mg) for 24 h. The mixture was diluted with water and filtered. The filtrate was concentrated, then lyophilised. The residue was dissolved in a solution of NaHCO<sub>3</sub> (75 mg) in water (1 mL) and purified by passing first through a column of C<sub>18</sub> silica (eluting with water), then through a column of Sephadex G<sub>10</sub> (eluting with water) to give, after lyophilisation, 518 (151 mg, 69%). HPLC (215 nm): Rt 4.09 min (Kromasil 5  $\mu$ m C<sub>18</sub> 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate). <sup>1</sup>H NMR (D<sub>2</sub>O):  $\delta$  4.97(d, 1H, J<sub>1,2</sub> = 3.8 Hz, H-1<sub>E</sub>), 4.78 (d, 1H, J<sub>1,2</sub> = 1.2 Hz, H-1<sub>C</sub>), 4.54 (d, 1H, J<sub>1,2</sub> = 8.6 Hz, H-1<sub>D</sub>), 4.02 (m, 1H, H-5<sub>C</sub>), 5.00-3.90 (m, 3H, H-5<sub>E</sub>, 6a<sub>D</sub>, CH<sub>2</sub>O), 3.88-3.67 (m, 7H, H-

2C, 2D, 3C, 6aE, 6bE, 6bD, CH<sub>2</sub>O), 3.61 (dd, 1H, *J* = 9.8, *J* = 9.1 Hz, H-3E), 3.60-3.42 (m, 5H, H-2E, 4C, 4D, 4E, 5D), 3.54 (m, 1H, H-3D), 3.03 (m, 2H, CH<sub>2</sub>NH<sub>2</sub>), 2.00 (s, 3H, CH<sub>3</sub>CO), 1.31 (d, 3H, *J*<sub>5,6</sub> = 6.3 Hz, H-6C); <sup>13</sup>C NMR (D<sub>2</sub>O): δ 175.2 (C=O), 101.6 (C-1C), 100.7 (C-1D), 100.0 (C-1E), 82.1 (C-3D), 81.4 (C-4C), 76.3 (C-2E), 73.1 (C-3E), 72.2 (C-5E), 71.9 (C-4D), 71.3 (C-2C), 69.7 (C-4E), 69.3 (C-3C), 68.8 (C-5D), 68.5 (C-5C), 66.0 (CH<sub>2</sub>O), 60.9 (C-6D), 60.5 (C-6E), 55.5 (C-2D), 39.8 (CH<sub>2</sub>NH<sub>2</sub>), 22.57 (CH<sub>3</sub>CO), 17.1 (C-6C). ES-MS for C<sub>22</sub>H<sub>40</sub>N<sub>2</sub>O<sub>15</sub> (572) *m/z* 573 [M+H]<sup>+</sup>. HRMS (MALDI) Calcd for C<sub>22</sub>H<sub>40</sub>N<sub>2</sub>O<sub>15</sub>Na: 595.2326. Found: 595.2341.

**Allyl (2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (521).** TMSOTf (100 μL) was added to a solution of donor **520** (2.5 g, 5.78 mmol) and acceptor **519** (4.0 g, 4.80 mmol) in Et<sub>2</sub>O (40 mL) at -50°C. The mixture was stirred for 2.5 h, at which time the cooling bath had reached rt. Et<sub>3</sub>N was added and after 15 min, volatiles were evaporated. Column chromatography (solvent C, 4:1) of the crude product gave the fully protected **521** (4.74 g, 89%) as a white solid. <sup>1</sup>H NMR: δ 8.00-6.90 (m, 25H, Ph), 5.92 (m, 1H, CH=), 5.53 (dd, 1H, H-2B), 5.40-5.20 (m, 4H, H-1E, 2C, CH<sub>2</sub>=), 5.18 (dd, 1H, *J*<sub>2,3</sub> = 3.4, *J*<sub>3,4</sub> = 10.0 Hz, H-3B), 5.10 (d, 1H, *J*<sub>1,2</sub> = 1.6 Hz, H-1B), 5.00-4.40 (m, 10H, H-4B, 1C, OCH<sub>2</sub>), 4.30-4.00 (m, 5H, H-3E, 3C, 5E, OCH<sub>2</sub>), 4.00-3.50 (m, 7H, H-2E, 4E, 6aE, 6bE, 5B, 5C, 4C), 1.90 (s, 3H, Ac), 1.60 (s, 3H, Ac), 1.22 (s, 3H, Ac), 1.20 (d, 3H, *J*<sub>5,6</sub> = 6.2 Hz Hz, H-6C), 0.80 (d, 3H, *J*<sub>5,6</sub> = 6.2 Hz, H-6B); <sup>13</sup>C NMR: δ 169.9, 169.7, 169.5, 166.1 (4C, C=O), 133.4-127.3 (Ph), 117.5 (=CH<sub>2</sub>), 9.8 (C-1B), 96.9 (C-1E), 95.7 (C-1C), 81.4 (C-3E), 80.7 (C-2E), 7.3 (C-3C), 77.7 (C-4E), 77.5 (brs, C-4C), 75.3, 74.6, 73.6 (3C, OCH<sub>2</sub>Ph), 72.8 (C-2C), 72.6 (CH<sub>2</sub>Ph), 70.9 (2C, C-5E, 4B), 69.6 (C-2B), 68.7 (C-6E), 68.6 (C-3B), 68.2 (OCH<sub>2</sub>), 67.2 (C-5C), 66.8 (C-5B), 20.7, 20.3, 20.2 (3C, C(O)CH<sub>3</sub>), 18.5 (C-6C), 16.8 (C-6B). CI-MS for C<sub>62</sub>H<sub>70</sub>O<sub>18</sub> (1102) *m/z* 1125 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>62</sub>H<sub>70</sub>O<sub>18</sub>: C, 67.50; H, 6.40%. Found: C, 67.51; H, 6.52%.

**(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranose (522).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (33 mg) was dissolved in THF (10 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, until the colour had changed to yellow. The solution was then degassed again in an argon stream. A solution of **521** (4.59 g, 4.16 mmol) in THF (30 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated. The residue was taken up in a mixture of acetone (10:1, 44 mL). Mercuric bromide (1.78 g, 8.32 mmol) and mercuric oxide (1.69 g, 6.24 mmol) were added to the mixture, which was protected from light. The suspension was stirred at rt for 1 h, then concentrated. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> and washed three times with sat aq KI, then with brine. The organic phase was dried and concentrated. The residue was

purified by column chromatography (solvent B, 3:1) to give **522** (3.52 g, 80%) as a colourless foam;  $^1\text{H}$  NMR:  $\delta$  7.15 (m, 25H, Ph), 5.50 (dd, 1H, H-2<sub>B</sub>), 5.30-5.27 (m, 2H, H-1<sub>C</sub>, H-2<sub>C</sub>), 5.23 (d, 1H,  $J_{1,2}$  = 3.3 Hz, H-1<sub>E</sub>), 5.18 (dd, 1H,  $J_{2,3}$  = 3.2,  $J_{3,4}$  = 10.0 Hz, H-3<sub>B</sub>), 5.10 (d, 1H,  $J_{1,2}$  = 1.2 Hz, H-1<sub>B</sub>), 5.00-4.35 (m, 9H, H-4<sub>B</sub>, OCH<sub>2</sub>), 4.28 (dd, 1H,  $J_{2,3}$  = 3.2,  $J_{3,4}$  = 8.6 Hz, H-3<sub>C</sub>), 4.20-4.00 (m, 3H, H-3<sub>E</sub>, 5<sub>E</sub>, 5<sub>C</sub>), 3.80-3.50 (m, 6H, H-2<sub>E</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 5<sub>B</sub>, 4<sub>E</sub>, 4<sub>C</sub>), 3.05 (d, 1H,  $J_{\text{OH},1}$  = 4.0 Hz, OH), 2.09, 1.81, 1.44 (3s, 9H, CH<sub>3</sub>C=O), 1.37 (d, 3H,  $J_{5,6}$  = 6.2 Hz, H-6<sub>C</sub>), 0.95 (d, 3H,  $J_{5,6}$  = 6.2 Hz, H-6<sub>B</sub>);  $^{13}\text{C}$  NMR:  $\delta$  169.9, 169.8, 169.6, 166.2 (4C, C=O), 138.9-127.5 (Ph), 99.8 (C-1<sub>B</sub>), 97.3 (C-1<sub>E</sub>), 91.3 (C-1<sub>C</sub>), 81.7 (C-3<sub>E</sub>), 80.7 (C-2<sub>E</sub>), 78.8 (C-3<sub>C</sub>), 78.1, 78.0 (2C, C-4<sub>E</sub>, 4<sub>C</sub>), 76.6, 75.5 (2C, CH<sub>2</sub>Ph), 74.9 (2C, C-2<sub>E</sub>, CH<sub>2</sub>Ph), 73.8 (CH<sub>2</sub>Ph), 73.3 (2C, C-4<sub>B</sub>, 5<sub>E</sub>), 72.9 (C-2<sub>B</sub>), 71.2 (2C, C-3<sub>B</sub>, 6<sub>E</sub>), 67.5 (C-5<sub>C</sub>), 67.1 (C-5<sub>B</sub>), 21.0, -20.6, 20.5 (3C, CH<sub>3</sub>C=O), 18.9 (C-6<sub>C</sub>), 17.1 (C-6<sub>B</sub>). FAB-MS for C<sub>59</sub>H<sub>66</sub>O<sub>18</sub> (1062)  $m/z$  1085 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>59</sub>H<sub>66</sub>O<sub>18</sub>·H<sub>2</sub>O: C, 65.54; H, 6.34%. Found: C, 65.68; H, 6.41%.

**(2,3,4-Tri-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranose trichloroacetimidate (**505**). DBU (100  $\mu\text{L}$ ) was added at 0°C to a solution of the hemiacetal **522** (3.8 g, 3.58 mmol) in DCM (40 mL) containing trichloroacetonitrile (4 mL). The mixture was stirred for 30 min at 0°C, and volatiles were evaporated. Flash chromatography (solvent B, 7:3 + 0.2% Et<sub>3</sub>N) of the crude material gave the donor **505** (3.9 g, 90%) as a white solid;  $^1\text{H}$  NMR ( $\alpha$  anomer):  $\delta$  8.75 (s, 1H, NH), 8.13-7.12 (m, 25H, Ph), 6.40 (d, 1H,  $J_{1,2}$  = 2.4 Hz, H-1<sub>C</sub>), 5.54 (br s, 1H, H-2<sub>B</sub>), 5.49 (dd, 1H,  $J_{2,3}$  = 2.9 Hz, H-2<sub>C</sub>), 5.26 (d, 1H,  $J_{1,2}$  = 2.8 Hz, H-1<sub>E</sub>), 5.20 (dd, 1H,  $J_{2,3}$  =  $J_{3,4}$  = 10.0 Hz, H-3<sub>B</sub>), 5.17 (br s, 1H, H-1<sub>B</sub>), 4.96 (dd, 1H, H-4<sub>B</sub>), 4.99-4.41 (m, 8H, OCH<sub>2</sub>), 4.34 (m, 1H, H-3<sub>C</sub>), 4.14-4.02 (m, 3H, H-3<sub>E</sub>, 5<sub>E</sub>, 5<sub>C</sub>), 3.87 (m, 1H, H-4<sub>C</sub>), 3.78 (dq, 1H,  $J_{4,5}$  = 9.5,  $J_{5,6}$  = 6.1 Hz, H-5<sub>B</sub>), 3.70 (m, 2H, H-6a<sub>E</sub>, 6b<sub>E</sub>), 3.65 (dd, 1H,  $J_{2,3}$  = 3.4,  $J_{3,4}$  = 9.8 Hz, H-2<sub>E</sub>), 3.57 (pt, 1H,  $J_{2,3}$  =  $J_{3,4}$  = 9.4 Hz, H-4<sub>E</sub>), 1.86, 1.83 (2s, 9H, CH<sub>3</sub>CO), 1.42 (d, 3H,  $J_{5,6}$  = 6.2 Hz, H-6<sub>C</sub>), 0.98 (d, 3H, H-6<sub>B</sub>);  $^{13}\text{C}$  NMR ( $\alpha$  anomer):  $\delta$  170.3, 170.1, 169.9, 166.1 (4C, C=O), 160.7 (C=NH), 139.2-127.8 (Ph), 100.2 (C-1<sub>B</sub>), 98.1 (C-1<sub>E</sub>), 94.8 (C-1<sub>C</sub>), 91.2 (CCl<sub>3</sub>), 82.4 (C-4<sub>C</sub>), 82.0 (C-3<sub>E</sub>), 81.2 (br s, C-2<sub>E</sub>), 78.5 (br s, C-3<sub>C</sub>), 78.3 (C-4<sub>E</sub>), 75.9, 75.4, 74.3, 73.3 (4C, CH<sub>2</sub>Ph), 71.8 (C-5<sub>E</sub>), 71.7 (C-2<sub>C</sub>), 71.3 (C-4<sub>B</sub>), 70.8 (br s, C-5<sub>C</sub>), 70.0 (C-2<sub>B</sub>), 69.3 (C-3<sub>B</sub>), 69.2 (C-6<sub>E</sub>), 67.6 (C-5<sub>B</sub>), 21.3, 21.0, 20.9 (3C, CH<sub>3</sub>CO), 18.9 (C-6<sub>C</sub>), 17.1 (C-6<sub>B</sub>). Anal. Calcd. for C<sub>61</sub>H<sub>66</sub>Cl<sub>3</sub>NO<sub>18</sub>: C, 60.67; H, 5.51; N, 1.16%. Found: C, 60.53; H, 5.48; N, 1.38%.**

**2-Azidoethyl (2,3,4-tri-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene- $\beta$ -D-glucopyranoside (**523**). The trisaccharide donor **505** (1.86 g, 1.54 mmol) and the acceptor **507** (712 mg, 2.16 mmol) were dissolved in 1,2-dichloroethane (15 mL) and 4Å-MS (2 g) were added. The mixture was stirred at rt for 1 h. The mixture was cooled to 0°C and triflic**

acid (34  $\mu$ L, 0.385 mmol) was added. The mixture was stirred at 0°C for 30 min, then at rt for 30 min. The mixture was then heated at 65°C for 1 h. The mixture was allowed to cool, Et<sub>3</sub>N (0.5 mL) was added, and the mixture was stirred at rt for 20 min. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 1:1) to give **523** (1.61 g, 76%). <sup>1</sup>H NMR:  $\delta$  7.90-6.90 (m, 25H, Ph), 5.92 (d, 1H,  $J = 7.5$  Hz, NH), 5.53 (dd, 1H,  $J_{1,2} = 1.8$  Hz, H-2<sub>B</sub>), 5.29 (d, 1H, H-1<sub>E</sub>), 5.19 (m, 2H, H-2<sub>C</sub>, 3<sub>B</sub>), 5.09 (m, 2H, H-1<sub>C</sub>, 1<sub>D</sub>), 4.97 (bs, 1H, H-1<sub>B</sub>), 4.96-4.70 (m, 9H, CH<sub>2</sub>Ph, H-4<sub>B</sub>), 4.54-4.41 (m, 1H, CH<sub>2</sub>Ph), 4.34 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.3$  Hz, H-3<sub>D</sub>), 4.19-3.89 (m, 6H, H-3<sub>C</sub>, 5<sub>C</sub>, 5<sub>E</sub>, 3<sub>E</sub>, 6a<sub>D</sub>, OCH<sub>2</sub>), 3.79-3.60 (m, 5H, H-6b<sub>D</sub>, 4<sub>C</sub>, 5<sub>B</sub>, 2<sub>E</sub>, OCH<sub>2</sub>), 3.56-3.33 (m, 4H, H-5<sub>D</sub>, 4<sub>E</sub>, 4<sub>D</sub>, CH<sub>2</sub>N<sub>3</sub>), 3.27-3.12 (m, 2H, CH<sub>2</sub>N<sub>3</sub>, H-2<sub>D</sub>), 2.10, 2.09 (2s, 6H, C(CH<sub>3</sub>)<sub>2</sub>), 1.78 (s, 3H, OAc), 1.73 (s, 3H, NHAc), 1.42, 1.35 (2s, 6H, OAc), 1.30 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>C</sub>), 0.90 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>B</sub>); <sup>13</sup>C NMR:  $\delta$  171.4, 169.7, 169.6, 169.5, 166.0 (5C, C=O), 138.7-127.2 (Ph), 99.8, 99.7 (C-1<sub>D</sub>, 1<sub>C</sub>), 97.1 (C-1<sub>B</sub>), 96.4 (C-1<sub>E</sub>), 81.5 (C-3<sub>E</sub>), 81.1 (C-2<sub>E</sub>), 79.5 (bs, C-3<sub>C</sub>), 77.9 (C-4<sub>D</sub>), 77.0 (bs, C-4<sub>C</sub>), 75.4 (C-3<sub>D</sub>), 75.3, 74.7, 73.6 (3C, CH<sub>2</sub>Ph), 73.0, 72.9 (2C, C-2<sub>C</sub>, 4<sub>E</sub>), 72.9 (CH<sub>2</sub>Ph), 71.2 (C-5<sub>E</sub>), 71.1 (C-4<sub>B</sub>), 69.9 (C-2<sub>B</sub>), 69.2 (C-6<sub>E</sub>), 68.8 (C-3<sub>B</sub>), 68.7 (OCH<sub>2</sub>), 67.2, 67.1 (3C, C-5<sub>C</sub>, 5<sub>B</sub>, 5<sub>D</sub>), 62.2 (C-6<sub>D</sub>), 59.0 (C-2<sub>D</sub>), 50.6 (CH<sub>2</sub>N<sub>3</sub>), 29.0, 23.4 (2C, C(CH<sub>3</sub>)<sub>2</sub>), 20.9, 20.4 (3C, OAc), 19.0 (NHAc), 18.4 (C-6<sub>C</sub>), 17.0 (C-6<sub>B</sub>). FAB-MS for C<sub>72</sub>H<sub>86</sub>N<sub>4</sub>O<sub>23</sub> (1374)  $m/z$  1397 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>72</sub>H<sub>86</sub>N<sub>4</sub>O<sub>23</sub>: C, 62.87; H, 6.30; N, 4.07%. Found: C, 63.51; H, 6.66; N, 3.77%.

**2-Azidoethyl (2,3,4-tri-*O*-acetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (**524**).** 50% aq TFA (1.3 mL) was added to a solution of the fully protected tetrasaccharide **523** (210 mg, 111  $\mu$ mol) in DCM (6 mL). The mixture was stirred at 0°C for 1 h. Volatiles were evaporated and toluene was co-evaporated from the residue. Column chromatography (solvent B, 7:3  $\rightarrow$  1:1) of the crude product gave **524** (195 mg, 95%). [ $\alpha$ ]<sub>D</sub> -6.9 ( $c$  0.5, MeOH); <sup>1</sup>H NMR:  $\delta$  8.08-7.14 (m, 25H, Ph), 5.78 (d, 1H,  $J_{2,NH} = 7.4$  Hz, NH), 5.51 (br s, 1H, H-2<sub>B</sub>), 5.27 (d, 1H,  $J_{1,2} = J_{2,3} = 2.9$  Hz, H-2<sub>C</sub>), 5.18 (m, 2H, H-1<sub>E</sub>, 3<sub>B</sub>), 5.12 (br s, 1H, H-1<sub>B</sub>), 5.08 (d, 1H,  $J_{1,2} = 8.3$  Hz, H-1<sub>D</sub>), 5.00 (d, 1H,  $J_{1,2} = 2.4$  Hz, H-1<sub>C</sub>), 4.97 (d, 1H,  $J = 11.0$  Hz, CH<sub>2</sub>Ph), 4.94 (pt, 1H,  $J_{3,4} = J_{4,5} = 9.9$  Hz, H-4<sub>B</sub>), 4.87-4.24 (m, 7H, CH<sub>2</sub>Ph), 4.21 (dd, 1H,  $J_{2,3} = 8.0$ ,  $J_{3,4} = 10.2$  Hz, H-3<sub>D</sub>), 4.19 (dd, 1H,  $J_{2,3} = 3.2$ ,  $J_{3,4} = 7.9$  Hz, H-3<sub>C</sub>), 4.10-4.04 (m, 2H, H-5<sub>C</sub>, 5<sub>E</sub>), 4.03 (pt, 1H,  $J_{2,3} = J_{3,4} = 9.4$  Hz, H-3<sub>E</sub>), 3.96 (dd, 1H,  $J_{5,6a} = 3.5$ ,  $J_{6a,6b} = 12.5$  Hz, H-6a<sub>D</sub>), 3.85 (dd, 1H,  $J_{5,6b} = 4.0$  Hz, H-6b<sub>D</sub>), 3.77-3.70 (m, 5H, H-4<sub>C</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, OCH<sub>2</sub>), 3.68 (m, 1H,  $J_{4,5} = 9.8$  Hz, H-5<sub>B</sub>), 3.63 (dd, 1H,  $J_{1,2} = 3.4$ ,  $J_{2,3} = 9.8$  Hz, H-2<sub>E</sub>), 3.60 (dd, 1H,  $J_{4,5} = 9.6$  Hz, H-4<sub>E</sub>), 3.55-3.44 (m, 3H, H-4<sub>D</sub>, 5<sub>D</sub>, CH<sub>2</sub>N<sub>3</sub>), 3.29 (m, 1H, CH<sub>2</sub>N<sub>3</sub>), 3.14 (m, 1H, H-2<sub>D</sub>), 2.13, 2.01, 1.82, 1.80 (4s, 12H, CH<sub>3</sub>CO), 1.39 (d, 3H,  $J_{5,6} = 6.2$  Hz, H-6<sub>C</sub>), 0.93 (d, 3H,  $J_{5,6} = 6.1$  Hz, H-6<sub>B</sub>); <sup>13</sup>C NMR:  $\delta$  171.5, 170.2, 170.1, 170.0,

166.3 (C=O), 139.2-127.9 (Ph), 99.8 (2C, C-1<sub>B</sub>, 1<sub>D</sub>), 99.5 (C-1<sub>C</sub>), 98.0 (br s, C-1<sub>E</sub>), 84.3 (C-3<sub>D</sub>), 82.0 (C-3<sub>E</sub>), 81.1 (C-2<sub>E</sub>), 78.8 (br s, C-3<sub>C</sub>), 78.2 (2C, C-4<sub>C</sub>, 4<sub>E</sub>), 75.9 (CH<sub>2</sub>Ph), 75.6 (C-4<sub>D</sub>), 75.2, 74.2, 73.4 (3C, CH<sub>2</sub>Ph), 73.0 (C-2<sub>C</sub>), 71.7 (C-5<sub>E</sub>), 71.4 (C-5<sub>D</sub>), 71.3 (C-4<sub>B</sub>), 70.1 (C-2<sub>B</sub>), 69.4 (C-6<sub>E</sub>), 69.2, 69.1 (C-3<sub>B</sub>, 5<sub>C</sub>), 68.9 (OCH<sub>2</sub>), 67.5 (C-5<sub>B</sub>), 63.2 (C-6<sub>D</sub>), 57.7 (C-2<sub>D</sub>), 51.1 (CH<sub>2</sub>N<sub>3</sub>), 23.8, 21.3, 21.0, 20.9 (4C, CH<sub>3</sub>CO), 19.1 (C-6<sub>C</sub>), 17.4 (C-6<sub>B</sub>). FAB-MS for C<sub>69</sub>H<sub>82</sub>N<sub>4</sub>O<sub>23</sub> (1334) *m/z* 1357.5 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>69</sub>H<sub>82</sub>N<sub>4</sub>O<sub>23</sub>·H<sub>2</sub>O: C, 60.43; H, 6.32; N, 4.09%. Found: C, 60.56; 6.22, 3.92%.

**2-Aminoethyl      α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside**

10 (525). An ice cold solution of 95% aqueous trifluoroacetic acid (2.4 mL) in CH<sub>2</sub>Cl<sub>2</sub> (21.6 mL) was added to the tetrasaccharide 523 (1.93 g, 1.40 mmol). The mixture was kept at 0°C for 5 min., then diluted with toluene and concentrated. Toluene was co-evaporated from the residue. The residue was dissolved in MeOH (65 mL), and a 1M solution of sodium methoxide in MeOH (3 mL) was added. The mixture was left to stand at rt for 18

15 h, then neutralised with Amberlite IR-120 (H<sup>+</sup>) resin, and filtered. The filtrate was concentrated, and the residue was purified by column chromatography (solvent B, 9:1) to give 524 (1.38 g, 89%) as a colourless foam. The tetrasaccharide 524 (1.38 g, 1.25 mmol) was dissolved in a mixture of EtOH (35 mL) and EtOAc (3.5 mL). A 1N solution of aq HCl (2.5 mL) was added. The mixture was stirred under hydrogen in the presence of 10%

20 Pd/C (1.5 g) for 72 h, then diluted with water and filtered. The filtrate was concentrated, then lyophilized. The residue was dissolved in a solution of 5% aq NaHCO<sub>3</sub> and purified by passing first through a column of C<sub>18</sub> silica (eluting with water), then through a column of Sephadex G<sub>10</sub> (eluting with water) to give, after lyophilization, 525 (693 mg, 77%). Further RP-HPLC purification of 373 mg of the latter gave 351 mg of RP-HPLC pure 525.

25 HPLC (215 nm): Rt 4.78 min (Kromasil 5 μm C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01 M aq TFA at 1 mL/min flow rate). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 5.10 (d, 1H, J<sub>1,2</sub> = 3.7 Hz, H-1<sub>E</sub>), 4.89 (d, 1H, J<sub>1,2</sub> = 1.1 Hz, H-1<sub>B</sub>), 4.73 (d, 1H, J<sub>1,2</sub> = 1.0 Hz, H-1<sub>C</sub>), 4.50 (d, 1H, J<sub>1,2</sub> = 8.6 Hz, H-1<sub>D</sub>), 4.08 (m, 1H, H-5<sub>C</sub>), 3.96 (m, 1H, H-2<sub>B</sub>), 3.91 (m, 2H, H-6a<sub>D</sub>, CH<sub>2</sub>O), 3.68-3.88 (m, 12H, H-2<sub>C</sub>, 2<sub>D</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 5<sub>B</sub>, 5<sub>E</sub>, 6b<sub>D</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, CH<sub>2</sub>O), 3.59 (pt, 1H, H-3<sub>E</sub>), 3.52 (pt, 1H, H-3<sub>D</sub>), 3.33-3.48 (m, 4H, H-2<sub>E</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>D</sub>), 3.01 (m, 2H, CH<sub>2</sub>NH<sub>2</sub>), 1.99 (s, 3H, CH<sub>3</sub>C=O), 1.28 (d, 3H, H-6<sub>C</sub>), 1.18 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR (D<sub>2</sub>O): δ 174.8 (C=O), 103.2 (C-1<sub>B</sub>), 101.4 (C-1<sub>C</sub>), 100.9 (C-1<sub>D</sub>), 98.6 (C-1<sub>E</sub>), 81.9 (C-3<sub>D</sub>), 79.0 (C-4<sub>B</sub>), 76.6 (C-4<sub>C</sub>), 76.3 (C-2<sub>E</sub>), 72.9 (C-3<sub>E</sub>), 72.3 (C-5<sub>E</sub>), 72.3 (C-4<sub>D</sub>), 71.8 (C-3<sub>C</sub>), 71.1 (C-2<sub>C</sub>), 70.5 (C-2<sub>B</sub>, 3<sub>B</sub>), 69.7 (C-4<sub>B</sub>), 69.5 (C-4<sub>E</sub>), 69.2 (C-5<sub>D</sub>), 68.8 (2C, C-5<sub>B</sub>, 5<sub>C</sub>), 67.9 (CH<sub>2</sub>O), 61.0 (C-6<sub>D</sub>), 60.8 (C-6<sub>E</sub>), 55.5 (C-2<sub>D</sub>), 40.0 (CH<sub>2</sub>NH<sub>2</sub>), 22.6 (CH<sub>3</sub>C=O), 18.0 (C-6<sub>C</sub>). 17.0 (C-6<sub>B</sub>). FAB-MS for C<sub>28</sub>H<sub>50</sub>N<sub>2</sub>O<sub>19</sub> (718) *m/z* 741 [M + Na]<sup>+</sup>. HRMS (MALDI) Calcd for C<sub>28</sub>H<sub>50</sub>N<sub>2</sub>O<sub>19</sub>Na: 741.2905. Found: 741.2939.

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**Allyl (2,3,4-Tri-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranoside (528).** TMSOTf (11  $\mu$ L, 59  $\mu$ mol) was added to a solution of the rhamnoside **526** (2.26 g, 5.88 mmol) and the trichloroacetimidate **527** (4.23 g, 6.82 mmol) in anhydrous Et<sub>2</sub>O (60 mL) at  $-70^{\circ}\text{C}$ . The reaction mixture was stirred for 8 h while the cooling bath was slowly coming back to rt. Et<sub>3</sub>N (100  $\mu$ L) was added, and the mixture was stirred at rt for 15 min. Solvents were evaporated, and the crude material was purified by column chromatography (solvent B, 49:1  $\rightarrow$  9:1), to give **528** as a white foam (4.78 g, 96%). <sup>1</sup>H NMR:  $\delta$  8.17-7.12 (m, 25H, Ph), 5.97-5.85 (m, 3H, H-2<sub>A</sub>, 3<sub>A</sub>, CH=), 5.67 (pt, 1H, J<sub>3,4</sub> = 9.6 Hz, H-4<sub>A</sub>), 5.34-5.19 (m, 3H, H-1<sub>A</sub>, CH<sub>2</sub>=), 5.01 (d, 1H, J = 9.0 Hz, CH<sub>2</sub>Ph), 4.92 (d, 1H, J<sub>1,2</sub> = 1.3 Hz, H-1<sub>B</sub>), 4.82-4.74 (m, 2H, CH<sub>2</sub>Ph), 4.71 (d, 1H, J = 11.8 Hz, OCH<sub>2</sub>), 4.31 (dq, 1H, J<sub>4,5</sub> = 9.7 Hz, H-5<sub>A</sub>), 4.21 (m, 1H, OCH<sub>2</sub>), 4.10 (dd, 1H, H-2<sub>B</sub>), 4.02 (m, 1H, OCH<sub>2</sub>), 3.97 (dd, 1H, J<sub>2,3</sub> = 3.0, J<sub>3,4</sub> = 9.2 Hz, H-3<sub>B</sub>), 3.82 (dq, 1H, J<sub>4,5</sub> = 9.4 Hz, H-5<sub>B</sub>), 3.71 (pt, 1H, H-4<sub>B</sub>), 1.43 (d, 3H, J<sub>5,6</sub> = 6.1 Hz, H-6<sub>B</sub>), 1.37 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>A</sub>); <sup>13</sup>C NMR:  $\delta$  166.3, 165.9, 165.7 (3C, C=O), 139.0-127.9 (CH=, Ph), 117.8 (CH<sub>2</sub>=), 99.9 (C-1<sub>A</sub>), 98.3 (C-1<sub>B</sub>), 80.6 (C-4<sub>B</sub>), 80.2 (C-3<sub>B</sub>), 76.5 (C-2<sub>B</sub>), 76.0, 72.9 (2C, CH<sub>2</sub>Ph), 72.3 (C-4<sub>A</sub>), 71.0 (C-2<sub>A</sub>\*), 70.4 (C-3<sub>A</sub>\*), 68.7 (C-5<sub>B</sub>), 68.1 (OCH<sub>2</sub>), 67.5 (C-5<sub>A</sub>), 18.4 (C-6<sub>B</sub>), 18.1 (C-6<sub>A</sub>). FAB-MS for C<sub>50</sub>H<sub>50</sub>O<sub>12</sub> (M, 842.3)  $m/z$  865.1 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>50</sub>H<sub>50</sub>O<sub>12</sub>: C, 71.24; H, 5.98%. Found C, 71.21; H, 5.99%.

**(2,3,4-tri-*O*-Benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranose (529).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (25 mg) was dissolved in THF (10 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, until the colour had changed to yellow. The solution was then degassed again in an argon stream. A solution of **528** (4.71 g, 5.59 mmol) in THF (40 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated. The residue was taken up in acetone (350 mL) and water (82 mL). Mercuric bromide (3.23 g, 8.96 mmol) and mercuric oxide (2.64 g, 12.3 mmol) were added to the mixture, which was protected from light. The suspension was stirred at rt for 1 h, then concentrated. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> and washed three times with sat aq KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 3:1) to give **529** (3.87 g, 84%) as a colourless foam. <sup>1</sup>H NMR:  $\delta$  8.15-7.12 (m, 25H, Ph), 5.94-5.88 (m, 3H, H-2<sub>A</sub>, 3<sub>A</sub>, CH=), 5.70 (pt, 1H, J<sub>3,4</sub> = 9.7 Hz, H-4<sub>A</sub>), 5.31 (dd, 1H, J<sub>1,OH</sub> = 3.0 Hz, H-1<sub>B</sub>), 5.28 (bs, 1H, H-1<sub>A</sub>), 4.98 (d, 1H, J = 11.0 Hz, CH<sub>2</sub>Ph), 4.82-4.68 (m, 3H, CH<sub>2</sub>Ph), 4.31 (dq, 1H, J<sub>4,5</sub> = 9.8 Hz, H-5<sub>A</sub>), 4.13 (dd, 1H, J<sub>1,2</sub> = 2.1 Hz, H-2<sub>B</sub>), 4.06-3.99 (m, 2H, H-3<sub>B</sub>, 5<sub>B</sub>), 3.72 (pt, 1H, J<sub>3,4</sub> = J<sub>4,5</sub> = 9.4 Hz, H-4<sub>B</sub>), 2.79 (bs, 1H, OH-1<sub>B</sub>), 1.41 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>B</sub>), 1.37 (d, 3H, J<sub>5,6</sub> = 6.3 Hz, H-6<sub>A</sub>); <sup>13</sup>C NMR:  $\delta$  166.2, 165.9, 165.7 (3C, C=O), 138.9-127.9 (Ph), 99.7 (C-1<sub>A</sub>), 94.2 (C-1<sub>B</sub>), 80.5 (C-4<sub>B</sub>), 79.6 (C-3<sub>B</sub>), 77.6 (C-2<sub>B</sub>), 76.5, 72.5 (2C, CH<sub>2</sub>Ph), 72.3 (C-4<sub>A</sub>), 71.0 (C-2<sub>A</sub>\*), 70.4 (C-3<sub>A</sub>\*), 68.8 (C-5<sub>B</sub>), 67.6 (C-5<sub>A</sub>), 18.5

(C-6<sub>B</sub>\*), 18.1 (C-6<sub>A</sub>\*). FAB-MS for C<sub>47</sub>H<sub>46</sub>O<sub>12</sub> (M, 802.3)  $m/z$  825.1 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>47</sub>H<sub>46</sub>O<sub>12</sub>·0.5 H<sub>2</sub>O: C, 69.53; H, 5.84%. Found C, 69.55; H, 5.76%.

**(2,3,4-Tri-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-3,4-di-*O*-benzyl- $\alpha/\beta$ -L-rhamnopyranosyl trichloroacetimidate (530).** The hemiacetal **529** (3.77 g, 4.71 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) and the solution was cooled to 0°C. Trichloroacetonitrile (2.5 mL) was added, then DBU (200  $\mu$ L). The mixture was stirred at rt for 2 h. Toluene was added, and co-evaporated twice from the residue. The crude material was purified by flash chromatography (solvent B, 4:1 + 0.1% Et<sub>3</sub>N) to give **530** as a white foam (4.29 g, 96%). Some hydrolyzed material **529** (121 mg, 3%) was eluted next.

The trichloroacetimidate **530**, isolated as an  $\alpha/\beta$  mixture had <sup>1</sup>H NMR ( $\alpha$  anomer):  $\delta$  8.62 (s, 1H, NH), 8.20-7.18 (m, 25H, Ph), 6.31 (s, 1H, H-1<sub>B</sub>), 5.94 (dd, 1H, J<sub>1,2</sub> = 1.6 Hz, H-2<sub>A</sub>), 5.89 (dd, 1H, J<sub>2,3</sub> = 3.4, J<sub>3,4</sub> = 9.9 Hz, H-3<sub>A</sub>), 5.71 (pt, 1H, H-4<sub>A</sub>), 5.27 (bs, 1H, H-1<sub>A</sub>), 5.02 (d, 1H, J = 10.8 Hz, CH<sub>2</sub>Ph), 4.84 (d, 1H, J = 11.9 Hz, CH<sub>2</sub>Ph), 4.79 (d, 1H, CH<sub>2</sub>Ph), 4.72 (d, 1H, CH<sub>2</sub>Ph), 4.36 (dq, 1H, J<sub>4,5</sub> = 9.8 Hz, H-5<sub>A</sub>), 4.13 (dd, 1H, H-2<sub>B</sub>), 4.03-3.97 (m, 2H, H-3<sub>B</sub>, 5<sub>B</sub>), 3.80 (pt, 1H, J<sub>3,4</sub> = 9.5 Hz, H-4<sub>B</sub>), 1.45 (d, 3H, J<sub>5,6</sub> = 6.1 Hz, H-6<sub>B</sub>), 1.40 (d, 3H, J<sub>5,6</sub> = 6.2 Hz, H-6<sub>A</sub>); <sup>13</sup>C NMR ( $\alpha$  anomer):  $\delta$  166.2, 165.9, 165.7 (3C, C=O), 160.8 (C=NH), 138.6-128.2 (Ph), 99.9 (C-1<sub>A</sub>), 97.2 (C-1<sub>B</sub>), 91.4 (CCl<sub>3</sub>), 79.9 (C-4<sub>B</sub>), 79.1 (C-3<sub>B</sub>), 76.2 (CH<sub>2</sub>Ph), 74.9 (C-2<sub>B</sub>), 73.3 (CH<sub>2</sub>Ph), 72.1 (C-4<sub>B</sub>), 71.7 (C-5<sub>B</sub>), 71.0 (C-2<sub>A</sub>), 70.2 (C-3<sub>A</sub>), 67.8 (C-5<sub>A</sub>), 18.4 (C-6<sub>B</sub>), 18.0 (C-6<sub>A</sub>). Anal. Calcd. for C<sub>49</sub>H<sub>46</sub>Cl<sub>3</sub>NO<sub>12</sub>: C, 62.13; H, 4.89; N, 1.48%. Found C, 61.81; H, 4.86; N, 1.36%.

**Allyl (2,3,4-Tri-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (533).** (a) The acceptor **519** (465 mg, 0.56 mmol) was dissolved in Et<sub>2</sub>O (3 mL). The solution was cooled to -60°C and TMSOTf (65  $\mu$ L, 0.36 mmol) was added. The donor **530** (690 mg, 0.73 mmol) was dissolved in Et<sub>2</sub>O (6 mL) and added to the acceptor solution in two portions with an interval of 30 min. The mixture was stirred at -60°C to -30°C over 2 h. Et<sub>3</sub>N (100  $\mu$ L) was added. The mixture was concentrated and the residue was purified by column chromatography (solvent B, 7:1) to give **533** (501 mg, 55%).

(b) A solution of the donor **527** (1.41 g, 2.25 mmol) and the acceptor **532** (1.07 g, 1.79 mmol) in anhydrous Et<sub>2</sub>O (88 mL) was cooled to -60°C. TMSOTf (63  $\mu$ L) was added, and the mixture was stirred at -60°C to -20°C over 2.5 h. Et<sub>3</sub>N was added (100  $\mu$ L). The mixture was concentrated and the residue was purified by column chromatography (solvent D, 49:1) to give **533** (2.66 g, 92%); [ $\alpha$ ]<sub>D</sub> +74.1 (c 0.5); <sup>1</sup>H NMR:  $\delta$  7.06-8.11 (m, 50H, Ph), 5.88-6.05 (m, 3H, H-2<sub>A</sub>, 3<sub>A</sub>, CH = ), 5.71 (t, 1H, H-4<sub>A</sub>), 5.51 (dd, 1H, H-2<sub>C</sub>), 5.22-5.41 (m, 3H, H-1<sub>A</sub>, CH<sub>2</sub> = ), 5.14 (d, 1H, J<sub>1,2</sub> = 0.9 Hz, H-1<sub>B</sub>), 5.10 (d, 1H, J<sub>1,2</sub> = 3.2 Hz, H-1<sub>E</sub>), 4.97 (bs, 1H, H-1<sub>C</sub>), 4.35-5.00 (m, 14H, H-2<sub>B</sub>, 5<sub>A</sub>, 12 x CH<sub>2</sub>Ph), 3.98-4.19 (m, 5H, H-3<sub>C</sub>, 3<sub>E</sub>, 5<sub>E</sub>, OCH<sub>2</sub>), 3.43-3.87 (m, 9H, H-2<sub>E</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>E</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 6<sub>E</sub>, 6'<sub>E</sub>),



1.44 (d, 3H, H-6<sub>A</sub>), 1.40 (d, 3H, H-6<sub>C</sub>), 1.13 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR: δ 165.9, 165.4, 165.1 (C=O), 127.1-138.7 (CH=, Ph), 117.8 (CH<sub>2</sub>=), 101.3 (C-1<sub>B</sub>), 99.6 (C-1<sub>A</sub>), 97.9 (C-1<sub>E</sub>), 96.1 (C-1<sub>C</sub>), 81.9 (C-3<sub>E</sub>), 81.0 (C-2<sub>E</sub>), 80.1 (C-3<sub>C</sub>), 79.8 (C-4<sub>B</sub>), 78.9 (C-3<sub>B</sub>), 77.9 (C-4<sub>C</sub>), 77.4 (C-4<sub>E</sub>), 75.9 (C-2<sub>B</sub>), 75.6, 75.0, 74.9, 73.9, 72.9 (CH<sub>2</sub>Ph), 72.4 (C-2<sub>C</sub>), 71.9 (C-4<sub>A</sub>), 71.2 (C-5<sub>E</sub>), 70.9 (CH<sub>2</sub>Ph), 70.7 (C-2<sub>A</sub>\*), 70.0 (C-3<sub>A</sub>\*), 69.2 (C-5<sub>B</sub>), 68.5 (OCH<sub>2</sub>), 68.1 (C-6<sub>E</sub>), 67.6 (C-5<sub>C</sub>), 67.2 (C-5<sub>A</sub>), 18.8 (C-6<sub>A</sub>), 18.1 (C-6<sub>C</sub>), 17.8 (C-6<sub>B</sub>). FAB-MS for C<sub>97</sub>H<sub>98</sub>O<sub>22</sub> (1614) *m/z* 1637 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>97</sub>H<sub>98</sub>O<sub>22</sub>: C, 72.10; H, 6.11%. Found: C, 71.75; H, 6.27%.

(2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-(2-*O*-benzoyl-α/β-L-rhamnopyranose (534). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (12.5 mg) was dissolved in THF (5 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of 533 (1.138 g, 0.70 mmol) in THF (15 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (7 mL) and water (0.7 mL). Mercuric chloride (285 mg, 1.05 mmol) and mercuric oxide (303 mg, 1.4 mmol) were added to the mixture, which was protected from light. The mixture was stirred at rt for 1 h, then concentrated. The residue was taken up in CH<sub>2</sub>Cl<sub>2</sub> and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 7:3) to give 534 (992 mg, 90%) as a colourless foam. <sup>1</sup>H NMR: δ 7.05-8.16 (m, 50H, Ph), 5.88-5.93 (m, 2H, H-2<sub>A</sub>, 3<sub>A</sub>), 5.73 (pt, 1H, H-4<sub>A</sub>), 5.55 (m, 1H, H-2<sub>C</sub>), 5.37 (bs, 1H, H-1<sub>A</sub>), 5.28 (bs, 1H, H-1<sub>C</sub>), 5.14 (bs, 1H, H-1<sub>B</sub>), 5.07 (d, 1H, J<sub>1,2</sub> = 3.1 Hz, H-1<sub>E</sub>), 4.78-4.99 (m, 6H, CH<sub>2</sub>Ph), 4.31-4.68 (m, 8H, H-2<sub>B</sub>, 5<sub>A</sub>, CH<sub>2</sub>Ph), 4.24 (dd, 1H, H-3<sub>C</sub>), 3.99-4.09 (m, 3H, H-3<sub>E</sub>, 5<sub>C</sub>, 5<sub>E</sub>), 3.82 (pt, 1H, H-4<sub>C</sub>), 3.57-3.76 (m, 5H, H-3<sub>B</sub>, 4<sub>E</sub>, 5<sub>B</sub>, 6<sub>A</sub>, 6<sub>E</sub>), 3.48 (dd, 1H, H-2<sub>E</sub>), 3.17 (d, 1H, OH), 1.43 (d, 6H, H-6<sub>A</sub>, 6<sub>C</sub>), 1.14 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR: δ 166.0, 165.6, 165.2 (4C, C=O), 127.2-138.9 (Ph), 101.1 (C-1<sub>B</sub>), 99.7 (C-1<sub>A</sub>), 98.1 (C-1<sub>E</sub>), 91.6 (C-1<sub>C</sub>), 81.9 (C-3<sub>E</sub>), 81.1 (C-2<sub>E</sub>), 79.9 (C-4<sub>B</sub>), 79.4 (C-3<sub>C</sub>), 78.9 (C-3<sub>B</sub>), 78.3 (C-4<sub>C</sub>), 77.6 (C-4<sub>E</sub>), 76.1 (C-2<sub>B</sub>), 75.8, 75.3, 75.1, 74.0, 73.1 (5C, CH<sub>2</sub>Ph), 72.7 (C-2<sub>C</sub>), 72.1 (C-4<sub>A</sub>), 71.4 (C-5<sub>E</sub>), 71.1 (CH<sub>2</sub>Ph), 70.8 (C-2<sub>A</sub>\*), 70.2 (C-3<sub>A</sub>\*), 69.4 (C-5<sub>B</sub>), 68.3 (C-6<sub>E</sub>), 67.7 (C-5<sub>C</sub>), 67.3 (C-5<sub>A</sub>), 19.0 (C-6<sub>A</sub>), 18.2 (C-6<sub>C</sub>), 17.9 (C-6<sub>B</sub>). FAB-MS for C<sub>94</sub>H<sub>94</sub>O<sub>22</sub> (1574) *m/z* 1597 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>94</sub>H<sub>94</sub>O<sub>22</sub>: C, 71.65; H, 6.01%. Found: C, 71.48; H, 6.17%.

(2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→4)]-(2-*O*-benzoyl-α/β-L-rhamnopyranosyl trichloroacetimidate (506). The hemiacetal 534 (412 mg, 0.26 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and the solution was cooled to 0°C.

Trichloroacetonitrile (0.26 mL) was added, then DBU (4  $\mu$ L). The mixture was stirred at 0°C for 1 h. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was purified by flash chromatography (solvent B, 4:1 + 0.1% Et<sub>3</sub>N) to give **506** (393 mg, 88%). <sup>1</sup>H NMR ( $\alpha$ -anomer):  $\delta$  8.74 (s, 1H, NH), 7.03-8.10 (m, 50H, Ph), 6.42 (d, 1H, J<sub>1,2</sub> = 2.3 Hz, H-1<sub>C</sub>), 5.87 (m, 2H, H-2<sub>A</sub>, 3<sub>A</sub>), 5.67 (m, 2H, H-2<sub>C</sub>, 4<sub>A</sub>), 5.30 (bs, 1H, H-1<sub>A</sub>), 5.14 (bs, 1H, H-1<sub>B</sub>), 5.08 (d, 1H, J<sub>1,2</sub> = 3.1 Hz, H-1<sub>E</sub>), 4.74-4.98 (m, 6H, CH<sub>2</sub>Ph), 4.23-4.69 (m, 9H, H-2<sub>B</sub>, 3<sub>C</sub>, 5<sub>A</sub>, CH<sub>2</sub>Ph), 3.88-4.07 (m, 3H, H-3<sub>E</sub>, 5<sub>B</sub>, 5<sub>E</sub>), 3.57-3.74 (m, 7H, H-2<sub>E</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>E</sub>, 5<sub>C</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>), 3.50 (dd, 1H, H-3<sub>B</sub>), 1.38 (d, 6H, H-6<sub>A</sub>, 6<sub>B</sub>), 1.07 (d, 3H, H-6<sub>C</sub>); <sup>13</sup>C NMR ( $\alpha$ -anomer):  $\delta$  165.9, 165.5, 165.4, 165.1 (4C, C=O), 160.1 (C=NH), 127.2-138.7 (Ph), 101.2 (C-1<sub>B</sub>), 99.7 (C-1<sub>A</sub>), 98.3 (C-1<sub>E</sub>), 94.3 (C-1<sub>C</sub>), 90.9 (CCl<sub>3</sub>), 81.7 (C-3<sub>E</sub>), 80.9 (C-2<sub>E</sub>), 79.6 (C-3<sub>C</sub>, 4<sub>B</sub>), 78.5 (C-3<sub>B</sub>), 77.2 (C-4<sub>C</sub>), 77.5 (C-4<sub>E</sub>), 75.9 (C-2<sub>B</sub>), 75.6, 75.1, 75.0, 74.0, 72.9 (CH<sub>2</sub>Ph), 71.8 (C-2<sub>C</sub>), 71.3 (C-4<sub>A</sub>), 71.0 (CH<sub>2</sub>Ph), 70.7 (C-5<sub>E</sub>), 70.5 (C-2<sub>A</sub>\*), 70.3 (C-3<sub>A</sub>\*), 70.0 (C-5<sub>B</sub>), 69.5 (C-5<sub>C</sub>), 67.9 (C-6<sub>E</sub>), 67.2 (C-5<sub>A</sub>), 18.7 (C-6<sub>A</sub>), 17.8 (C-6<sub>C</sub>), 17.7 (C-6<sub>B</sub>). Anal. Calcd. for C<sub>96</sub>H<sub>94</sub>Cl<sub>3</sub>NO<sub>22</sub>·H<sub>2</sub>O: C, 66.34; H, 5.57; N, 0.81%. Found: C, 66.26, H, 5.72; N, 0.94%.

**2-Azidoethyl (2,3,4-tri-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl)-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene- $\beta$ -D-glucopyranoside (**535**). (a) The tetrasaccharide donor **506** (500 mg, 0.29 mmol) and the acceptor **507** (140 mg, 0.42 mmol) were dissolved in 1,2-dichloroethane (5 mL) and 4Å-MS (400 mg) were added. The mixture was stirred at rt for 2 h. The mixture was cooled to 0°C and triflic acid (7  $\mu$ L, 0.072 mmol) was added. The mixture was stirred at 0°C to rt over 1 h 30 min. The mixture was then heated at 65°C for 1 h 30 min. The mixture was allowed to cool, Et<sub>3</sub>N (0.5 mL) was added, and the mixture was stirred at rt for 20 min. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 4:3) to give **535** (340 mg, 62%).**

(b) The tetrasaccharide donor **506** (250 mg, 145  $\mu$ mol) and the acceptor **507** (67 mg, 204  $\mu$ mol) were dissolved in DCM (1.5 mL) and 4Å-MS (200 mg) were added. The mixture was stirred at -40°C for 30 min and triflic acid (5  $\mu$ L) was added. The mixture was stirred at rt over 3 h, triethylamine was added, and the mixture was stirred at rt for 15 min. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 9:1  $\rightarrow$  1:1) to give **535** (219 mg, 80%). [ $\alpha$ ]<sub>D</sub> +64.0 (c 0.1); <sup>1</sup>H NMR:  $\delta$  7.04-8.06 (m, 50H, Ph), 6.24 (d, 1H, NH), 5.90 (m, 2H, H-2<sub>A</sub>, 3<sub>A</sub>), 5.70 (t, 1H, H-4<sub>A</sub>), 5.42 (m, 1H, H-2<sub>C</sub>), 5.35 (bs, 1H, H-1<sub>A</sub>), 5.13 (m, 3H, H-1<sub>B</sub>, 1<sub>D</sub>, 1<sub>E</sub>), 4.77-5.00 (m, 5H, H-1<sub>C</sub>, CH<sub>2</sub>Ph), 4.29-4.66 (m, 11H, H-2<sub>B</sub>, 3<sub>D</sub>, 5<sub>A</sub>, CH<sub>2</sub>Ph), 3.80-4.11 (m, 6H, H-3<sub>C</sub>, 3<sub>E</sub>, 5<sub>C</sub>, 5<sub>E</sub>, 6<sub>aD</sub>, CH<sub>2</sub>O), 3.45-3.78 (m, 12H, H-2<sub>E</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>B</sub>, 5<sub>D</sub>, 6<sub>bD</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>, CH<sub>2</sub>O), 3.39 (m, 1H, CH<sub>2</sub>N<sub>3</sub>), 3.23 (m, 2H, H-2<sub>D</sub>,

CH<sub>2</sub>N<sub>3</sub>), 2.13 (s, 3H, CH<sub>3</sub>CO), 1.43 (d, 9H, H-6<sub>A</sub>, (CH<sub>3</sub>)<sub>2</sub>C), 1.29 (d, 3H, H-6<sub>C</sub>), 1.11 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR: δ 171.8, 165.9, 165.5, 165.0, 163.5 (5C, C=O), 127.1-138.7 (Ph), 101.3 (C-1<sub>B</sub>), 99.8 (C-1<sub>D</sub>), 99.3 (C-1<sub>A</sub>), 97.7 (C-1<sub>C</sub>), 97.6 (C-1<sub>E</sub>), 91.8 (C(CH<sub>3</sub>)<sub>2</sub>), 81.6 (C-3<sub>E</sub>), 81.0 (C-2<sub>E</sub>), 80.0 (C-3<sub>C</sub>), 79.7 (C-4<sub>D</sub>), 78.9 (C-4<sub>B</sub>), 77.5 (C-3<sub>B</sub>, 4<sub>C</sub>), 76.4 (C-3<sub>D</sub>), 75.6 (C-2<sub>B</sub>), 75.5, 74.9, 74.8, 73.8, 73.0 (5C, CH<sub>2</sub>Ph), 72.9 (C-4<sub>E</sub>), 72.7 (C-2<sub>C</sub>), 71.8 (C-4<sub>A</sub>), 71.3 (C-5<sub>E</sub>), 71.0 (CH<sub>2</sub>Ph), 70.6 (C-2<sub>A</sub>\*), 70.0 (C-3<sub>A</sub>\*), 69.3 (C-5<sub>B</sub>), 68.6 (OCH<sub>2</sub>), 68.3 (C-6<sub>E</sub>), 67.5 (C-5<sub>C</sub>), 67.3 (C-5<sub>A</sub>), 67.1 (C-5<sub>D</sub>), 62.2 (C-6<sub>D</sub>), 58.9 (C-2<sub>D</sub>), 50.6 (CH<sub>2</sub>N<sub>3</sub>), 29.1 (CH<sub>3</sub>C), 23.6 (CH<sub>3</sub>C=O), 19.2 (CH<sub>3</sub>C), 18.6 (C-6<sub>A</sub>), 18.0 (C-6<sub>C</sub>), 17.6 (C-6<sub>B</sub>). FAB-MS for C<sub>107</sub>H<sub>114</sub>N<sub>4</sub>O<sub>27</sub> (1886) *m/z* 1909 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>107</sub>H<sub>114</sub>N<sub>4</sub>O<sub>27</sub>: C, 68.07, H, 6.09; N, 2.97%. Found: C, 68.18, H, 6.07; N, 2.79%.

**2-Aminoethyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (537).** An ice cold solution of 95% aq TFA (2.1 mL) in CH<sub>2</sub>Cl<sub>2</sub> (8 mL) was added to the pentasaccharide **535** (283 mg, 0.15 mmol). The mixture was kept at 0°C for 2 h, then diluted with toluene and concentrated. Toluene was co-evaporated from the residue. Chromatography of the residue (solvent B, 7:3 → 1:1) gave the intermediate diol (265 mg, 96%). The latter (265 mg) was dissolved in MeOH (6 mL), and a 1 % solution of methanolic sodium methoxide (4.0 mL) was added. The mixture was stirred at 55°C for 2 h, then neutralised with Dowex X8 (H<sup>+</sup>) resin and filtered. The filtrate was concentrated. The mixture was purified by column chromatography (solvent A, 100:0 → 95:5) to give **536** (195 mg, 87%) as a colourless foam, whose structure was confirmed from mass spectrometry analysis (FAB-MS for C<sub>76</sub>H<sub>94</sub>N<sub>4</sub>O<sub>23</sub> (M, 1430) *m/z* 1453 [M + Na]<sup>+</sup>). Pentasaccharide **536** (171 mg, 0.11 mmol) was dissolved in EtOH (18 mL). A 1 M solution of aq HCl (210 μL) was added. The mixture was stirred under hydrogen in the presence of 10% Pd/C (96 mg) for 2 h. The mixture was diluted with EtOH and water, then filtered through a pad of Celite. The filtrate was concentrated, and preliminary purified by passing first through a column of C<sub>18</sub> silica (eluting with water). The residue was purified by RP-HPLC to give, after lyophilization, **537** (50 mg, 53%). HPLC (215 nm): Rt 5.87 min (Kromasil 5 μm C<sub>18</sub> 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 5.15 (d, 1H, J<sub>1,2</sub> = 3.7 Hz, H-1<sub>E</sub>), 5.00 (bs, 1H, H-1<sub>A</sub>), 4.92 (d, 1H, J<sub>1,2</sub> = 1.1 Hz, H-1<sub>B</sub>), 4.76 (bs, 1H, H-1<sub>C</sub>), 4.53 (d, 1H, J<sub>1,2</sub> = 8.6 Hz, H-1<sub>D</sub>), 4.10 (m, 1H, H-5<sub>C</sub>), 4.03 (m, 2H, H-2<sub>A</sub>, 2<sub>B</sub>), 4.01 (m, 3H, H-4<sub>A</sub>, 4<sub>B</sub>, CH<sub>2</sub>O), 3.83-3.88 (m, 7H, H-2<sub>C</sub>, 2<sub>D</sub>, 3<sub>A</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>, 6<sub>aE</sub>, CH<sub>2</sub>O), 3.69-3.76 (m, 7H, H-3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>C</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 6<sub>bE</sub>), 3.52 (pt, 1H, H-3<sub>D</sub>), 3.33-3.54 (m, 5H, H-2<sub>E</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>D</sub>, 5<sub>E</sub>), 3.09 (m, 2H, CH<sub>2</sub>NH<sub>2</sub>), 1.98 (s, 3H, CH<sub>3</sub>C=O), 1.28 (d, 3H, H-6<sub>C</sub>), 1.22 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR (D<sub>2</sub>O): δ 175.3 (C=O), 103.4 (C-1<sub>B</sub>), 101.9 (C-1<sub>A</sub>), 101.4 (C-1<sub>C</sub>, 1<sub>D</sub>), 98.4 (C-1<sub>E</sub>), 82.3 (C-3<sub>D</sub>), 80.2 (C-2<sub>B</sub>), 79.9, 76.7 (C-2<sub>E</sub>), 72.9, 72.4, 72.4, 72.2, 71.8, 71.6, 70.5, 70.4, 70.1, 70.0, 69.7, 69.6, 69.4, 68.7, 66.7 (CH<sub>2</sub>O), 61.0 (2C, C-6<sub>D</sub>, 6<sub>E</sub>), 55.5

(C-2<sub>D</sub>), 39.9 (CH<sub>2</sub>NH<sub>2</sub>), 22.6 (CH<sub>3</sub>C=O), 18.2 (C-6<sub>C</sub>), 17.2 (C-6<sub>A</sub>), 17.0 (C-6<sub>B</sub>). HRMS (MALDI) Calcd for C<sub>34</sub>H<sub>60</sub>N<sub>2</sub>O<sub>23</sub>+H: 865.3665. Found: 865.3499.

**Maleimido activated PADRE Lys (508).**

Starting from 0.1 mmol of Fmoc Pal Peg Ps resin, amino acids (0.4 mmol) were incorporated using HATU/DIEA (0.4 mmol) activation. The N-terminal D-Ala was incorporated as Boc-D-Ala-OH. After completion of the chain elongation, the resin was treated three times with hydrazine monohydrate (2% solution in DMF, 25 mL/g of peptide resin) for 3min, which allowed the selective deblocking of the Dde protecting group. To a solution of maleimide butyric acid (183 mg, 1.0 mmol) in DCM (2 mL) was added DCC (103 mg, 0.5 mmol). After stirring for 10 min, the suspension was filtered, and the filtrate was added to the drained peptide resin. DIEA (17 µL, 0.5 mmol) was added. After 30 min, the peptide resin was washed with DMF (100 mL), MeOH (100 mL), and dried under vacuum. After TFA/TIS/H<sub>2</sub>O (95/2.5/2.5) cleavage (10 mL/g of resin, 1.5 h), the crude peptide (157 mg) was dissolved in 16 mL of 15% CH<sub>3</sub>CN in 0.08% aq TFA, and purified by reverse phase Medium Pressure Liquid Chromatography (MPLC) on a Nucleoprep 20 µm C18 100 Å column, using a 15-75% linear gradient of CH<sub>3</sub>CN in 0.08% aq TFA over 60 min at 25 mL/min flow rate (214 nm detection) to give **508** (107 mg, 61%). HPLC (214 nm): Rt 13.4 min (94% pure, Nucleosil 5 µm C18 300 Å analytical column, using a 15-45% linear gradient over 20 min of CH<sub>3</sub>CN in 0.08% aq TFA at 25 mL/min flow rate). Positive ion ES-MS Calcd for C<sub>85</sub>H<sub>139</sub>N<sub>21</sub>O<sub>19</sub>: 1759.18. Found: 1758.83 (SD: 0.40).

**(S-Acetylthiomethyl)carbonylaminoethyl α-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (538).** The trisaccharide **518** (58 mg, 0.1 mmol) was dissolved in DMF (1 mL). SAMA-Pfp (33 mg, 0.11 mmol) was added, and the mixture was left to stand at rt for 40 min. Toluene was added and the mixture was concentrated. Ether was added to the residue. The resulting precipitate was collected and purified by passing through a column of C<sub>18</sub> silica (water-acetonitrile, gradient) to give **538** (36 mg, 53%). HPLC (230 nm): Rt 13.74 min (99% pure, Kromasil 5 µm C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate). <sup>13</sup>C NMR (D<sub>2</sub>O): δ 200.3 (SC=O), 175.2, 171.9 (NC=O), 102.1 (C-1<sub>C</sub>), 101.2 (C-1<sub>D</sub>), 100.5 (C-1<sub>E</sub>), 82.7 (C-3<sub>D</sub>), 81.8 (C-4<sub>C</sub>), 76.8 (C-2<sub>E</sub>), 73.6 (C-3<sub>E</sub>), 72.6 (C-5<sub>E</sub>), 72.4 (C-4<sub>D</sub>), 71.8 (C-2<sub>C</sub>), 70.2 (C-4<sub>E</sub>), 69.7 (C-3<sub>C</sub>), 69.4 (C-5<sub>D</sub>), 68.9 (C-5<sub>C</sub>), 68.9 (CH<sub>2</sub>O), 61.6 (C-6<sub>D</sub>), 60.9 (C-6<sub>E</sub>), 56.1 (C-2<sub>D</sub>), 40.6 (CH<sub>2</sub>NH), 33.7 (CH<sub>2</sub>S), 30.4 (CH<sub>3</sub>C(O)S), 23.0 (CH<sub>3</sub>C(O)N), 17.5 (C-6<sub>C</sub>). ES-MS for C<sub>26</sub>H<sub>44</sub>N<sub>2</sub>O<sub>17</sub>S (688) *m/z* 689 [M+H]<sup>+</sup>. HRMS (MALDI) Calcd for C<sub>22</sub>H<sub>44</sub>N<sub>2</sub>O<sub>17</sub>SNa: 711.2258. Found: 711.2294.

**(S-Acetylthiomethyl)carbonylaminoethyl α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-**

**deoxy- $\beta$ -D-glucopyranoside (539).** A solution of SAMA-Pfp (16.7 mg, 40  $\mu$ mol) in CH<sub>3</sub>CN (150  $\mu$ L) was added to the tetrasaccharide **525** (20 mg, 28.8  $\mu$ mol) in 0.1 M phosphate buffer (pH 7.4, 600  $\mu$ L). The mixture was stirred at rt for 45 min and purified by RP-HPLC to give **539** (17 mg, 74%). HPLC (230 nm): Rt 13.63 min (98% pure, Kromasil 5  $\mu$ m C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate). <sup>1</sup>H NMR (D<sub>2</sub>O):  $\delta$  5.10 (d, 1H,  $J_{1,2}$  = 3.7 Hz, H-1<sub>E</sub>), 4.91 (d, 1H,  $J_{1,2}$  = 0.8 Hz, H-1<sub>B</sub>), 4.73 (bs, 1H, H-1<sub>C</sub>), 4.45 (d, 1H,  $J_{1,2}$  = 8.5 Hz, H-1<sub>D</sub>), 4.09 (m, 1H, H-5<sub>C</sub>), 3.97 (m, 1H, H-2<sub>B</sub>), 3.87 (m, 4H, H-2<sub>C</sub>, 3<sub>C</sub>, 6a<sub>D</sub>, CH<sub>2</sub>O), 3.62-3.78 (m, 8H, H-2<sub>D</sub>, 3<sub>B</sub>, 4<sub>C</sub>, 5<sub>B</sub>, 6b<sub>D</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 1 x CH<sub>2</sub>O), 3.60 (m, 3H, H-3<sub>E</sub>, CH<sub>2</sub>S), 3.48 (pt, 1H, H-3<sub>D</sub>), 3.39-3.46 (m, 6H, H-2<sub>E</sub>, 4<sub>B</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>D</sub>, 5<sub>E</sub>), 3.33 (m, 2H, CH<sub>2</sub>NH<sub>2</sub>), 2.35 (s, 3H, CH<sub>3</sub>C(O)S), 1.98 (s, 3H, CH<sub>3</sub>C(O)N), 1.27 (d, 3H, H-6<sub>C</sub>), 1.23 (d, 3H, H-6<sub>B</sub>); <sup>13</sup>C NMR (D<sub>2</sub>O):  $\delta$  199.8 (SC=O), 174.5, 171.3 (NC(O)), 103.2 (C-1<sub>B</sub>), 101.4 (C-1<sub>C</sub>), 100.9 (C-1<sub>D</sub>), 98.6 (C-1<sub>E</sub>), 82.0 (C-3<sub>D</sub>), 79.0 (C-4<sub>B</sub>), 76.6 (C-4<sub>C</sub>), 76.3 (C-2<sub>E</sub>), 72.9 (C-3<sub>E</sub>), 72.3 (C-5<sub>E</sub>), 72.2 (C-4<sub>D</sub>), 71.8 (C-3<sub>C</sub>), 71.0 (C-2<sub>C</sub>), 70.5 (C-2<sub>B</sub>, 3<sub>B</sub>), 69.7 (C-4<sub>B</sub>), 69.5 (C-4<sub>E</sub>), 69.1 (C-5<sub>C</sub>, 5<sub>D</sub>), 68.8 (C-5<sub>B</sub>), 68.7 (CH<sub>2</sub>O), 61.1 (C-6<sub>D</sub>), 60.7 (C-6<sub>E</sub>), 55.5 (C-2<sub>D</sub>), 40.1 (CH<sub>2</sub>NH), 33.2 (CH<sub>2</sub>S), 29.9 (CH<sub>3</sub>C(O)S), 22.6 (CH<sub>3</sub>C(O)N), 17.9 (C-6<sub>C</sub>), 16.9 (C-6<sub>B</sub>). MS for C<sub>32</sub>H<sub>54</sub>N<sub>2</sub>O<sub>21</sub>S (834)  $m/z$  857 [M + Na]<sup>+</sup>. HRMS-MALDI Calcd for C<sub>32</sub>H<sub>54</sub>N<sub>2</sub>O<sub>21</sub>S+Na: 857.2838. Found: 857.2576.

**(S-Acetylthiomethyl)carbonylaminoethyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (540).** The pentasaccharide **537** (6.4 mg, 7.4  $\mu$ mol) was dissolved in 0.1 M phosphate buffer (pH 7.4, 1.0 mL). SAMA-Pfp (6.6 mg, 22  $\mu$ mol) was added, and the mixture was stirred at rt for 5 h. More SAMA-Pfp (6.6 mg, 22  $\mu$ mol) was added and the mixture was stirred for 1 h more at rt. RP-HPLC of the mixture gave **540** (5.4 mg, 75%). HPLC (230 nm): Rt 13.86 min (100% pure, Kromasil 5  $\mu$ m C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate). <sup>1</sup>H NMR (D<sub>2</sub>O):  $\delta$  5.13 (d, 1H,  $J_{1,2}$  = 3.7 Hz, H-1<sub>E</sub>), 4.98 (bs, 1H, H-1<sub>A</sub>), 4.90 (bs, 1H, H-1<sub>B</sub>), 4.74 (bs, 1H, H-1<sub>C</sub>), 4.47 (d, 1H,  $J_{1,2}$  = 8.5 Hz, H-1<sub>D</sub>), 4.09 (m, 1H, H-5<sub>C</sub>), 4.00 (m, 2H, H-2<sub>A</sub>, 2<sub>B</sub>), 3.79-3.85 (m, 8H, H-2<sub>C</sub>, 2<sub>D</sub>, 3<sub>A</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>, CH<sub>2</sub>O), 3.65-3.74 (m, 9H, H-3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>C</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, CH<sub>2</sub>O), 3.60 (m, 2H, CH<sub>2</sub>S), 3.53 (pt, 1H, H-3<sub>D</sub>), 3.13-3.49 (m, 7H, H-2<sub>E</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>D</sub>, 5<sub>E</sub>, CH<sub>2</sub>NH), 2.35 (s, 3H, CH<sub>3</sub>C=OS), 1.99 (s, 3H, CH<sub>3</sub>C=ON), 1.28 (d, 3H, H-6<sub>C</sub>), 1.20 (m, 6H, H-6<sub>A</sub>, 6<sub>B</sub>); <sup>13</sup>C NMR (D<sub>2</sub>O):  $\delta$  199.9 (SC=O), 174.5, 171.4 (NC=O), 102.8 (C-1<sub>B</sub>), 101.7 (C-1<sub>A</sub>), 101.4 (C-1<sub>C</sub>), 100.9 (C-1<sub>D</sub>), 97.9 (C-1<sub>E</sub>), 82.0 (C-3<sub>D</sub>), 79.7 (C-2<sub>B</sub>), 79.0, 76.3, 72.9, 72.4, 72.2, 71.8, 71.0, 70.5, 69.7, 69.5, 69.1, 68.8, 68.5 (CH<sub>2</sub>O), 61.2, 61.0 (2C, C-6<sub>D</sub>, 6<sub>E</sub>), 55.6 (C-2<sub>D</sub>), 40.1 (CH<sub>2</sub>NH), 33.2 (CH<sub>2</sub>S), 29.9 (CH<sub>3</sub>C=OS), 22.7 (CH<sub>3</sub>C=ON), 18.2 (C-6<sub>C</sub>), 17.2 (C-6<sub>A</sub>), 17.0 (C-6<sub>B</sub>). HRMS (MALDI) Calcd for C<sub>38</sub>H<sub>64</sub>N<sub>2</sub>O<sub>25</sub>SNa: 1003.3417. Found: 1003.3426.

**PADRE-Lys-(thiomethyl)carbonylaminoethyl  $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (501).**

Compound 538 (5.0 mg, 7.3  $\mu$ mol) was dissolved in water (500  $\mu$ L) and added to a solution of 508 (10 mg, 5.68  $\mu$ mol) in a mixture of water (900  $\mu$ L), acetonitrile (100  $\mu$ L) and 0.1M phosphate buffer (pH 6.0, 1 mL). 117  $\mu$ L of a solution of hydroxylamine hydrochloride (139 mg/mL) in 0.1M phosphate buffer (pH 6.0) was added and the mixture was stirred for 1 h. RP-HPLC purification gave the pure glycopeptide 501 (8.5 mg, 62%). HPLC (230 nm): Rt 10.40 min (100% pure, Kromasil 5  $\mu$ m C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01 M aq TFA at 1 mL/min flow rate). ES-MS Calcd for C<sub>109</sub>H<sub>181</sub>N<sub>23</sub>O<sub>35</sub>S: 2405.85. Found: 2405.52.

**PADRE-Lys-(thiomethyl)carbonylaminoethyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (502).** Compound 539 (4.9 mg, 5.8  $\mu$ mol) was dissolved in water (500  $\mu$ L) and added to a solution of 508 (13 mg, 7.4  $\mu$ mol) in a mixture of water (1 mL), acetonitrile (200  $\mu$ L) and 0.5 M phosphate buffer (pH 5.7, 1.2 mL). 117  $\mu$ L of a solution of hydroxylamine hydrochloride (139 mg/mL) in 0.5M phosphate buffer (pH 5.7) was added, and the mixture was stirred for 1 h. RP-HPLC purification gave the pure glycopeptide 502 (6.7 mg, 48%). HPLC (230 nm): Rt 11.60 min (100% pure, Kromasil 5  $\mu$ m C18 100 Å 4.6x250 mm analytical column, using a 20-50% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate). ES-MS Calcd for C<sub>125</sub>H<sub>191</sub>N<sub>23</sub>O<sub>39</sub>S: 2552.99. Found: 2551.90.

**PADRE-Lys-(thiomethyl)carbonylaminoethyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (503).** Compound 540 (5.59 mg, 5.7  $\mu$ mol) was dissolved in water (500  $\mu$ L) and added to a solution of 508 (12.6 mg, 7.2  $\mu$ mol) in a mixture of water (1 mL), acetonitrile (200  $\mu$ L), which had been previously diluted with 0.5 M phosphate buffer (pH 5.7, 1.2 mL). A solution of hydroxylamine hydrochloride (139 mg/mL) in 0.5M phosphate buffer (pH 5.7, 117  $\mu$ L) was added and the mixture was stirred for 1 h. RP-HPLC purification gave the pure glycopeptide 503 (7.1 mg, 46%). HPLC (230 nm): Rt 10.33 min (100% pure, Kromasil 5  $\mu$ m C18 100 Å 4.6x250 mm analytical column, using a 20-50% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01 M aq TFA at 1 mL/min flow rate). ES-MS Calcd for C<sub>121</sub>H<sub>201</sub>N<sub>23</sub>O<sub>43</sub>S: 2698.14. Found: 2698.09.

**F- Synthesis of two linear PADRE-conjugates bearing a deca- or a pentasaccharide B epitope as potential synthetic vaccine against *Shigella flexneri* serotype 2a infection**

**Allyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-**

**rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1→4)]-2-*O*-**

**benzoyl- $\alpha$ -L-rhamnopyranoside (611).** A mixture of **610** (3.14 g, 1.6 mmol), Bu<sub>3</sub>SnH (2.5 mL, 9.3 mmol) and AIBN (240 mg) in dry toluene (40 mL) was stirred for 30 min at rt under a stream of dry Argon, then was heated for 1 h at 100°C, cooled and concentrated.

- 5 The residue was eluted from a column of silica gel with 3:2 petroleum ether-EtOAc to give **611** as a white foam (2.0 g, 68 %);  $[\alpha]_D +3^\circ$  (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.00-7.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.58 (d, 1H,  $J_{2,NH} = 8.0$  Hz, N-H<sub>D</sub>), 5.35 (dd, 1H,  $J_{1,2} = 1.0$ ,  $J_{2,3} = 2.3$  Hz, H-2<sub>C</sub>), 5.19 (m, 2H, All), 5.10 (d, 1H,  $J_{1,2} = 1.0$  Hz, H-1<sub>A</sub>), 4.92 (dd, 1H,  $J_{2,3} = 10.5$ ,  $J_{3,4} = 10.5$  Hz, H-3<sub>D</sub>), 4.92 (d, 1H,  $J_{1,2} = 3.3$  Hz, H-1<sub>E</sub>), 4.90 (d, 1H,  $J_{1,2} = 1.7$  Hz, H-1<sub>B</sub>), 4.89 (d, 1H, H-1<sub>C</sub>), 4.88 (dd, 1H,  $J_{4,5} = 10.0$  Hz, H-4<sub>D</sub>), 4.62 (d, 1H,  $J_{1,2} = 8.5$  Hz, H-1<sub>D</sub>), 4.90-4.35 (m, 16H, CH<sub>2</sub>Ph), 4.40 (m, 1H, H-2<sub>B</sub>), 4.10-4.00 (m, 2H, All), 4.08 (dd, 1H,  $J_{2,3} = 2.4$  Hz, H-2<sub>A</sub>), 4.02 (dd, 1H, H-3<sub>C</sub>), 3.91 (m, 1H, H-2<sub>D</sub>), 3.90-3.70 (m, 11H, H-4<sub>C</sub>, 5<sub>C</sub>, 3<sub>A</sub>, 5<sub>A</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>, 3<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>), 3.61 (dd, 1H,  $J_{3,4} = 9.5$  Hz, H-3<sub>B</sub>), 3.55 (m, 1H, H-5<sub>B</sub>), 3.41-3.40 (m, 3H, H-4<sub>A</sub>, 5<sub>D</sub>, 2<sub>E</sub>), 3.47 (m, 1H,  $J_{4,5} = 9.5$ ,  $J_{5,6} = 6.1$  Hz, H-5<sub>B</sub>), 3.35-3.33 (m, 3H, H-4<sub>A</sub>, 5<sub>D</sub>, 2<sub>E</sub>), 3.25 (dd, 1H, H-4<sub>B</sub>), 1.95, 1.70 (3s, 9H, OAc), 1.65 (s, 3H, NHAc), 1.32 (d, 3H,  $J_{5,6} = 6.1$  Hz, H-6<sub>A</sub>), 1.30 (d, 3H,  $J_{5,6} = 6.0$  Hz, H-6<sub>C</sub>), 0.97 (d, 3H,  $J_{5,6} = 6.0$  Hz, H-6<sub>B</sub>). <sup>13</sup>C NMR:  $\delta$  171.1, 170.8, 170.2, 169.6, 166.2 (5C, C=O), 138.2-118.5 (Ph, All), 103.1 (C-1<sub>D</sub>), 101.4 (C-1<sub>B</sub>), 101.2 (C-1<sub>A</sub>), 98.5 (C-1<sub>E</sub>), 96.4 (C-1<sub>C</sub>), 82.2 (C-3<sub>E</sub>), 81.7 (C-2<sub>E</sub>), 81.7 (C-4<sub>A</sub>), 80.4 (C-4<sub>B</sub>), 80.2 (C-3<sub>C</sub>), 79.0 (C-3<sub>A</sub>), 78.6 (C-3<sub>B</sub>), 78.1 (C-2<sub>A</sub>), 77.8 (C-4<sub>C</sub>), 77.6 (C-4<sub>E</sub>), 76.0, 75.8, 75.4, 74.7, 74.3, 74.2, 73.3, 70.5 (8C, CH<sub>2</sub>Ph), 74.9 (C-2<sub>B</sub>), 72.7 (C-2<sub>C</sub>), 72.6 (C-3<sub>D</sub>), 71.9 (2C, C-5<sub>E</sub>, 5<sub>D</sub>), 69.1 (C-5<sub>B</sub>), 68.9 (2C, All, C-5<sub>A</sub>), 68.3 (C-6<sub>E</sub>), 67.8 (C-5<sub>C</sub>), 62.3 (C-6<sub>D</sub>), 54.6 (C-2<sub>D</sub>), 23.5 (NHAc), 21.1, 21.0, 20.8 (3C, OAc), 19.0 (C-6<sub>C</sub>), 18.4 (C-6<sub>A</sub>), 18.2 (C-6<sub>B</sub>). FAB-MS of C<sub>104</sub>H<sub>117</sub>NO<sub>27</sub> (M, 1913.1), *m/z* 1936.2 [M+Na]<sup>+</sup>. Anal. Calcd. for C<sub>104</sub>H<sub>117</sub>NO<sub>27</sub>: C, 68.90; H, 6.50; N, 0.77. Found:
- 25 C, 68.64; H, 6.66; N, 1.05.

**Allyl****(2-acetamido-4,6-*O*-isopropylidene-2-deoxy- $\beta$ -D-**

**glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1**

**→4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (613).** The pentasaccharide **611** (2.65 g, 1.47 mmol) was dissolved in MeOH (20 mL). MeONa was added until pH 10. The mixture was stirred for 25 min then treated by IR 120 (H<sup>+</sup>) until neutral pH. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with 9:1 DCM-MeOH to give the expected triol **612** which was then treated overnight at rt by 2,2-dimethoxypropane (11 mL, 0.1 mol) and PTSA (20 mg, 0.17 mmol) in DMF (20 mL).

- 35 Et<sub>3</sub>N was added and the solution evaporated. The residue was eluted from a column of silica gel with 1:1 cyclohexane-EtOAc and 0.2 % of Et<sub>3</sub>N to give **613** as a white foam (2.05 g, 81 % from **611**);  $[\alpha]_D +3^\circ$  (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  6.98-8.00 (m, 45H, Ph), 6.17 (bs, 1H, NH<sub>D</sub>), 5.82 (m, 1H, All), 5.30 (dd, 1H,  $J_{1,2} = 1.0$ ,  $J_{2,3} = 3.0$  Hz, H-2<sub>C</sub>), 5.11-5.25

(m, 2H, All), 5.06 (bs, 1H, H-1<sub>A</sub>), 4.92 (d, 1H,  $J_{1,2} = 3.1$  Hz, H-1<sub>E</sub>), 4.88 (d, 1H,  $J_{1,2} = 1.6$  Hz, H-1<sub>B</sub>), 4.84 (bs, 1H, H-1<sub>C</sub>), 4.35 (d, 1H, H-1<sub>D</sub>), 4.34 (dd, 1H, H-2<sub>B</sub>), 4.20-4.80 (m, 16H, CH<sub>2</sub>Ph), 4.05 (dd, 1H, H-2<sub>A</sub>), 3.36 (dd, 1H, H-2<sub>E</sub>), 2.90-4.10 (m, 22H, All, H-2<sub>D</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>D</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>D</sub>, 5<sub>E</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>), 1.5 (s, 3H, NHAc), 1.2-0.9 (m, 15H, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR:  $\delta$  172.7, 164.9 (2C, C=O), 137.7-116.7 (Ph, All), 102.3 (C-1<sub>D</sub>), 100.2 (C-1<sub>B</sub>), 100.0 (C-1<sub>A</sub>), 98.9 (C(CH<sub>3</sub>)<sub>2</sub>), 97.2 (C-1<sub>E</sub>), 95.1 (C-1<sub>C</sub>), 82.1, 82.0, 81.8, 81.6, 80.6, 80.3, 79.0, 78.8, 78.3, 77.8, 77.6, 75.7, 75.6, 75.0, 74.3, 72.8, 71.8, 71.6, 70.8, 70.3, 69.0, 68.5, 67.8, 67.4, 61.9, 60.8, 60.5, 29.4 (C(CH<sub>3</sub>)<sub>2</sub>), 22.7 (NHAc), 19.0 (C(CH<sub>3</sub>)<sub>2</sub>), 18.9, 18.4, 18.2 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). FAB-MS for C<sub>101</sub>H<sub>115</sub>NO<sub>24</sub> (M, 1726.9)  $m/z$  1749.7 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>101</sub>H<sub>115</sub>NO<sub>24</sub>·H<sub>2</sub>O: C, 69.52; H, 6.76; N, 0.80. Found: C, 69.59; H 6.71; N, 0.57.

**Allyl (2-acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranoside (614).** A mixture of 613 (2.05 g, 1.19 mmol) in pyridine (60 mL) was cooled to 0°C. Acetic anhydride (20 mL) was added and the solution was stirred 2.5 h. The solution was concentrated and coevaporated with toluene. The residue was eluted from a column of silica gel with 2:1 Cyclohexane-EtOAc and 0.2 % of Et<sub>3</sub>N to give 614 as a white foam (1.99 g, 94 %);  $[\alpha]_D^{+10}$  (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  6.95-8.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.46 (d, 1H,  $J_{2,NH} = 8.0$  Hz, NH<sub>D</sub>), 5.29 (dd, 1H,  $J_{1,2} = 1.0$ ,  $J_{2,3} = 3.0$  Hz, H-2<sub>C</sub>), 5.11-5.25 (m, 2H, All), 5.00 (bs, 1H, H-1<sub>A</sub>), 4.90 (d, 1H,  $J_{1,2} = 3.1$  Hz, H-1<sub>E</sub>), 4.85 (d, 1H,  $J_{1,2} = 1.6$  Hz, H-1<sub>B</sub>), 4.83 (bs, 1H, H-1<sub>C</sub>), 4.70 (dd, 1H,  $J_{2,3} = J_{3,4} = 10.0$  Hz, H-3<sub>D</sub>), 4.44 (d, 1H, H-1<sub>D</sub>), 4.34 (dd, 1H, H-2<sub>B</sub>), 4.20-4.80 (m, 16H, CH<sub>2</sub>Ph), 4.02 (dd, 1H, H-2<sub>A</sub>), 3.37 (dd, 1H, H-2<sub>E</sub>), 2.90-4.10 (m, 21H, All, H-2<sub>D</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>D</sub>, 5<sub>E</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>), 1.92 (s, 3H, OAc), 1.57 (s, 3H, NHAc), 1.27-0.90 (m, 15H, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR:  $\delta$  171.3, 170.3, 166.2 (3C, C=O), 138.7-117.9 (Ph, All), 103.9 (C-1<sub>D</sub>), 101.5 (C-1<sub>B</sub>), 101.4 (C-1<sub>A</sub>), 99.9 (C(CH<sub>3</sub>)<sub>2</sub>), 98.5 (C-1<sub>E</sub>), 96.3 (C-1<sub>C</sub>), 82.1, 81.7, 81.6, 80.3, 80.1, 78.8, 78.1, 77.8, 76.0, 75.8, 75.3, 75.1, 74.7, 74.2, 73.6, 73.3, 72.7, 71.9, 71.4, 70.8, 69.0, 68.8, 68.7, 68.4, 68.1, 67.8, 62.1, 55.0 (C-2<sub>D</sub>), 30.0 (C(CH<sub>3</sub>)<sub>2</sub>), 23.5 (NHAc), 21.6 (OAc), 19.2 (C(CH<sub>3</sub>)<sub>2</sub>), 19.0, 18.3, 18.2 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). FAB-MS for C<sub>103</sub>H<sub>117</sub>NO<sub>25</sub> (M, 1769.0)  $m/z$  1791.9 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>103</sub>H<sub>117</sub>NO<sub>25</sub>: C, 69.93; H, 6.67; N, 0.79. Found: C, 69.77; H, 6.84; N, 0.72.

**(2-Acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl trichloroacetimidate (607).** 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (50 mg, 58  $\mu$  mol) was dissolved THF (10 mL), and the resulting red solution was degassed in an argon



stream. Hydrogen was then bubbled through the solution, causing the color to change to yellow. The solution was then degassed again in an argon stream. A solution of **614** (1.8 g, 1.02 mmol) in THF (20 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated to dryness. The residue was dissolved in acetone (9 mL), then water (2 mL), mercuric chloride (236 mg) and mercuric oxide (200 mg) were added successively. The mixture protected from light was stirred at rt for 2 h, and acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aq KI, water and satd aq NaCl, dried and concentrated. The residue was eluted from a column of silica gel with 3:2 Cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give the corresponding hemiacetal **615**. Trichloroacetonitrile (2.4 mL) and DBU (72  $\mu$ L) were added to a solution of the residue in anhydrous DCM (24 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 3:2 cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give **607** as a colourless oil (1.58 g, 82 % from **614**); [ $\alpha$ ]<sub>D</sub> +2° (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  8.62 (s, 1H, NH), 6.95-8.00 (m, 45H, Ph), 6.24 (d, 1H,  $J_{1,2}$  = 2.6 Hz, H-1<sub>C</sub>), 5.48 (dd, 1H,  $J_{2,3}$  = 3.0 Hz, H-2<sub>C</sub>), 5.41 (d, 1H,  $J_{2,NH}$  = 8.4 Hz, NH<sub>D</sub>), 4.99 (bs, 1H, H-1<sub>A</sub>), 4.92 (d, 1H,  $J_{1,2}$  = 3.2 Hz, H-1<sub>E</sub>), 4.88 (d, 1H,  $J_{1,2}$  = 1.6 Hz, H-1<sub>B</sub>), 4.69 (dd, 1H,  $J_{2,3}$  =  $J_{3,4}$  = 10.0 Hz, H-3<sub>D</sub>), 4.44 (d, 1H, H-1<sub>D</sub>), 4.34 (dd, 1H, H-2<sub>B</sub>), 4.20-4.80 (m, 16H, CH<sub>2</sub>Ph), 4.02 (dd, 1H, H-2<sub>A</sub>), 3.38 (dd, 1H, H-2<sub>E</sub>), 2.90-4.10 (m, 19H, H-2<sub>D</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>D</sub>, 4<sub>E</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>D</sub>, 5<sub>E</sub>, 6a<sub>D</sub>, 6b<sub>D</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>), 1.95 (s, 3H, OAc), 1.55 (s, 3H, NHAc), 1.30-0.85 (m, 15H, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR:  $\delta$  172.4, 171.4, 166.9 (3C, C=O), 140.2-128.9 (Ph), 104.2 (C-1<sub>D</sub>), 101.4 (2C, C-1<sub>A</sub>, 1<sub>B</sub>), 101.1 (C(CH<sub>3</sub>)<sub>2</sub>), 98.0 (C-1<sub>E</sub>), 94.8 (C-1<sub>C</sub>), 92.4 (CCl<sub>3</sub>), 82.1, 81.5, 80.2, 80.1, 78.6, 78.1, 77.8, 77.6, 76.0, 75.8, 75.5, 75.0, 74.3, 74.2, 73.5 (C-3<sub>D</sub>), 73.4, 71.9, 71.4, 71.0, 70.5, 69.2, 68.8, 68.3, 68.1, 62.1, 54.9 (C-2<sub>D</sub>), 29.3 (C(CH<sub>3</sub>)<sub>2</sub>), 23.4 (NHAc), 21.4 (OAc), 19.2 (C(CH<sub>3</sub>)<sub>2</sub>), 19.0, 18.2, 18.1 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). FAB-MS for C<sub>102</sub>H<sub>113</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>25</sub> (M, 1873.3)  $m/z$  1896.3 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>102</sub>H<sub>113</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>25</sub>: C, 65.40; H, 6.08; N, 1.50. Found: C, 65.26; H, 6.02; N, 1.31.

**2-Azidoethyl (2-acetamido-3-O-acetyl-2-deoxy-4,6-O-isopropylidene- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-O-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-4,6-O-isopropylidene- $\beta$ -D-glucopyranoside (**616**).** A mixture of donor **607** (745 mg, 0.4 mmol) and acceptor **608** (170 mg, 0.51 mmol), 4 Å molecular sieves and dry 1,2-DCE (12 mL), was stirred for 1 h then cooled to 0°C. Triflic acid (25  $\mu$ L) was added. The stirred mixture was allowed to reach rt in 10 min then stirred again for 2.5 h at 75°C. After cooling to rt, Et<sub>3</sub>N (100  $\mu$ L) was added and the mixture filtered. After evaporation, the residue was eluted from a column of silica gel with 1:2 cyclohexane-EtOAc and 0.2 % Et<sub>3</sub>N to give **616** as a white foam (615 mg, 76 %); [ $\alpha$ ]<sub>D</sub> +0° (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  6.95-7.90 (m, 45H, Ph), 6.02 (d, 1H,  $J_{2,NH}$  = 7.1 Hz, NH<sub>D</sub>), 5.46 (d, 1H,  $J_{2,NH}$  = 8.6 Hz, NH<sub>D'</sub>), 5.20 (dd,

1H,  $J_{1,2} = 1.0$ ,  $J_{2,3} = 3.0$  Hz, H-2<sub>C</sub>), 5.03 (d, 1H,  $J_{1,2} = 8.1$  Hz, H-1<sub>D</sub>), 5.02 (bs, 1H, H-1<sub>A</sub>), 4.92 (d, 1H,  $J_{1,2} = 3.1$  Hz, H-1<sub>E</sub>), 4.85 (d, 1H,  $J_{1,2} = 1.6$  Hz, H-1<sub>B</sub>), 4.82 (bs, 1H, H-1<sub>C</sub>), 4.70 (dd, 1H, H-3<sub>D'</sub>), 4.44 (d, 1H, H-1<sub>D'</sub>), 4.30 (dd, 1H, H-2<sub>B</sub>), 4.20-4.80 (m, 16H, CH<sub>2</sub>Ph), 3.99 (dd, 1H, H-2<sub>A</sub>), 3.37 (dd, 1H, H-2<sub>E</sub>), 2.90-3.95 (m, 29H, H-2<sub>D</sub>, 2<sub>D'</sub>, 3<sub>A</sub>, 3<sub>B</sub>, 3<sub>C</sub>, 3<sub>D</sub>, 3<sub>E</sub>, 4<sub>A</sub>, 4<sub>B</sub>, 4<sub>C</sub>, 4<sub>D</sub>, 4<sub>D'</sub>, 4<sub>E</sub>, 5<sub>A</sub>, 5<sub>B</sub>, 5<sub>C</sub>, 5<sub>D</sub>, 5<sub>D'</sub>, 5<sub>E</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>, 6<sub>aD'</sub>, 6<sub>bD'</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>, OCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 2.00 (s, 3H, NHAc), 1.92 (s, 3H, OAc), 1.57 (s, 3H, NHAc), 1.27-0.90 (m, 21H, 2 C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR: δ 172.1, 171.5, 170.3, 166.2 (4C, C=O), 139.0-127.7 (Ph), 103.9 (C-1<sub>D'</sub>), 101.7 (C-1<sub>B</sub>), 101.2 (C-1<sub>A</sub>), 100.0 (C-1<sub>D</sub>), 99.9, 99.8 (2C, C(CH<sub>3</sub>)<sub>2</sub>), 98.3 (C-1<sub>E</sub>), 97.8 (C-1<sub>C</sub>), 82.0, 81.7, 81.5, 80.8, 80.2, 80.1, 78.9, 78.6, 78.0, 77.9, 76.0, 75.9, 75.8, 75.3, 74.8, 74.6, 74.2, 74.0, 73.6, 73.5, 73.4, 73.0, 71.9, 71.4, 70.8, 69.1, 69.0, 68.8, 68.6, 68.0, 67.7, 67.6, 62.6, 62.1, 60.8, 59.7 (C-2<sub>D</sub>), 55.0 (C-2<sub>D'</sub>), 51.1 (CH<sub>2</sub>N<sub>3</sub>), 29.5 (C(CH<sub>3</sub>)<sub>2</sub>), 29.3 (C(CH<sub>3</sub>)<sub>2</sub>), 23.9 (NHAc), 23.5 (NHAc), 21.3 (OAc), 19.7 (C(CH<sub>3</sub>)<sub>2</sub>), 19.2 (C(CH<sub>3</sub>)<sub>2</sub>), 18.8, 18.4, 18.2 (3C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). FAB-MS for C<sub>113</sub>H<sub>133</sub>N<sub>5</sub>O<sub>30</sub> (M, 2041.3)  $m/z$  2064.2 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>113</sub>H<sub>133</sub>N<sub>5</sub>O<sub>30</sub>: C, 66.49; H, 6.57; N, 3.43. Found: C, 65.93; H, 6.57; N, 2.61.

**2-Azidoethyl (2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (617).** The hexasaccharide **616** (615 mg, 0.30 mmol) was dissolved in MeOH (8 mL). MeONa was added until pH 9. The mixture was stirred for 3 h, then treated by IR 120 (H<sup>+</sup>) until neutral pH. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with 25:1 DCM-MeOH and 0.2 % of Et<sub>3</sub>N to give **617** as a white foam (590 mg, 97 %); [α]<sub>D</sub> +1° (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR: δ 8.00-7.00 (m, 45H, Ph), 6.10 (d, 1H, NH<sub>D</sub>), 6.05 (d, 1H,  $J_{2,NH} = 7.4$  Hz, NH<sub>D</sub>), 5.20 (dd, 1H,  $J_{1,2} = 1.7$ ,  $J_{2,3} = 3.0$  Hz, H-2<sub>C</sub>), 5.10 (d, 1H,  $J_{1,2} = 1.0$  Hz, H-1<sub>A</sub>), 4.99 (d, 1H,  $J_{1,2} = 8.3$  Hz, H-1<sub>D</sub>), 4.96 (d, 1H,  $J_{1,2} = 3.2$  Hz, H-1<sub>E</sub>), 4.90 (d, 1H,  $J_{1,2} = 1.0$  Hz, H-1<sub>B</sub>), 4.86 (d, 1H,  $J_{1,2} = 1.0$  Hz, H-1<sub>C</sub>), 4.52 (d, 1H,  $J_{1,2} = 7.5$  Hz, H-1<sub>D'</sub>), 4.37 (dd, 1H, H-2<sub>B</sub>), 4.22 (dd, 1H, H-3<sub>D</sub>), 4.02 (dd, 1H, H-2<sub>A</sub>), 4.80-4.00 (m, 16H, CH<sub>2</sub>Ph), 4.00-2.95 (m, 30H, H-2<sub>D</sub>, 4<sub>D</sub>, 5<sub>D</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>, 2<sub>E</sub>, 3<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>, 3<sub>C</sub>, 4<sub>C</sub>, 5<sub>C</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 5<sub>B</sub>, 3<sub>A</sub>, 4<sub>A</sub>, 5<sub>A</sub>, 2<sub>D'</sub>, 3<sub>D'</sub>, 4<sub>D'</sub>, 5<sub>D'</sub>, 6<sub>aD'</sub>, 6<sub>bD'</sub>, OCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 2.00-0.92 (6s, 3d, 27H, NHAc, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR partial: δ 173.9, 172.1, 166.3 (3C, C=O), 140.0-125.0 (Ph), 103.6 (C-1<sub>D'</sub>), 101.7 (C-1<sub>B</sub>), 101.2 (C-1<sub>A</sub>), 100.2 (C(CH<sub>3</sub>)<sub>2</sub>), 100.2 (C-1<sub>D</sub>), 99.9 (C(CH<sub>3</sub>)<sub>2</sub>), 98.2 (C-1<sub>E</sub>), 97.8 (C-1<sub>C</sub>), 51.1 (CH<sub>2</sub>N<sub>3</sub>), 29.4, 29.3, 23.9, 22.8, 19.6, 19.2, 18.9, 18.4, 18.2 (C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, NHAc, C(CH<sub>3</sub>)<sub>2</sub>). FAB-MS for C<sub>111</sub>H<sub>131</sub>N<sub>5</sub>O<sub>29</sub> (M, 1999.2)  $m/z$  2021.8 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>111</sub>H<sub>131</sub>N<sub>5</sub>O<sub>29</sub>: C, 66.68; H, 6.60; N, 3.50. Found: C, 66.63; H, 6.78; N, 3.32.

**(2-*O*-Acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-**

(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (606). 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (80 mg, 93 μmol) was dissolved in THF (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of 609 (2.55 g, 1.67 mmol) in THF (20 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated to dryness. The residue was dissolved in acetone (15 mL), then water (3 mL), mercuric chloride (380 mg) and mercuric oxide (320 mg) were added successively. The mixture protected from light was stirred at rt for 2 h, and acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aq KI, water and satd aq NaCl, dried and concentrated. The residue was eluted from a column of silica gel with 3:1 petroleum ether-EtOAc to give the corresponding hemiacetal. Trichloroacetonitrile (2.0 mL) and DBU (25 μL) were added to a solution of the residue in anhydrous DCM (15 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with 3:1 petroleum ether-EtOAc and 0.2 % Et<sub>3</sub>N to give 606 as a white foam (1.5 g, 56 %); [α]<sub>D</sub> +22° (c 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR: δ 8.72 (s, 1H, C=NH), 8.00-7.00 (m, 45H, Ph), 6.39 (d, 1H, *J*<sub>1,2</sub> = 2.5 Hz, H-1<sub>C</sub>), 5.60 (dd, 1H, *J*<sub>2,3</sub> = 3.0 Hz, H-2<sub>C</sub>), 5.58 (dd, 1H, *J*<sub>1,2</sub> = 1.7 Hz, *J*<sub>2,3</sub> = 3.0 Hz, H-2<sub>A</sub>), 5.12 (d, 1H, *J*<sub>1,2</sub> = 3.2 Hz, H-1<sub>E</sub>), 5.08 (m, 2H, H-1<sub>A</sub>, 1<sub>B</sub>), 5.00-4.00 (m, 16H, CH<sub>2</sub>Ph), 4.20 (dd, 1H, H-3<sub>C</sub>), 4.05 (dd, 1H, H-3<sub>E</sub>), 4.00-3.35 (m, 14H, H-2<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>, 6a<sub>E</sub>, 6b<sub>E</sub>, 4<sub>C</sub>, 5<sub>C</sub>, 2<sub>B</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 5<sub>B</sub>, 3<sub>A</sub>, 4<sub>A</sub>, 5<sub>A</sub>), 2.05 (s, 3H, OAc), 1.42, 1.36 and 1.00 (3d, 9H, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). <sup>13</sup>C NMR: δ 170.3, 165.8 (2C, C=O), 138-127 (Ph), 99.9 (2C, C-1<sub>A</sub>, 1<sub>B</sub>), 98.5 (C-1<sub>E</sub>), 94.7 (C-1<sub>C</sub>), 82.1, 81.2, 80.4, 80.0, 79.1, 78.1, 78.0, 75.2, 71.7, 71.2, 70.7, 69.5, 69.4, 68.7 (16C, C-2<sub>A</sub>, 3<sub>A</sub>, 4<sub>A</sub>, 5<sub>A</sub>, 2<sub>B</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 5<sub>B</sub>, 2<sub>C</sub>, 3<sub>C</sub>, 4<sub>C</sub>, 5<sub>C</sub>, 2<sub>E</sub>, 3<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>), 76.0, 75.7, 75.5, 75.1, 74.3, 73.3, 72.2, 71.2 (8C, PhCH<sub>2</sub>), 68.5 (C-6<sub>E</sub>), 21.4 (OAc), 19.2, 18.5, 18.1 (C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>). Anal. Calcd. for C<sub>91</sub>H<sub>96</sub>Cl<sub>3</sub>NO<sub>20</sub>: C, 67.05; H, 5.94; N, 0.86. Found: C, 66.44; H, 6.21; N, 0.93.

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (618). A mixture of alcohol 617 (110 mg, 55 μmol), trichloroacetimidate 606 (179 mg, 110 μmol) and 4Å molecular sieves in anhydrous 1,2-DCE (2.5 mL) was stirred for 1 h under dry Ar. After cooling at -35°C, triflic acid (5 μL, 50 μmol) was added dropwise and the mixture was stirred for 2.5 h, while allowed to reach 10°C. Et<sub>3</sub>N (25 μL) was added, and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel

with 4:1 to 3:1 toluene-EtOAc and Et<sub>3</sub>N (0.2 %) to give **618** as a white foam (158 mg, 82 %); [ $\alpha$ ]<sub>D</sub> +18° (*c* 1, CHCl<sub>3</sub>). <sup>1</sup>H NMR:  $\delta$  8.00-6.90 (90H, m, Ph), 5.90 (d, 1H,  $J_{2,NH}$  = 7.0 Hz, NH<sub>D</sub>), 5.58 (d, 1H,  $J_{2,NH}$  = 7.5 Hz, NH<sub>D'</sub>), 5.45, 5.22 (m, 2H,  $J_{1,2}$  = 1.0,  $J_{2,3}$  = 2.0 Hz, H-2<sub>C</sub>, 2<sub>C'</sub>), 5.12 (dd, 1H, H-2<sub>A'</sub>), 5.11 (d, 1H,  $J_{1,2}$  = 8.3 Hz, H-1<sub>D</sub>), 5.05 (d, 1H,  $J_{1,2}$  = 1.0 Hz, H-1<sub>A</sub>), 5.01 (d, 1H,  $J_{1,2}$  = 3.2 Hz, H-1<sub>E</sub>), 4.96 (d, 1H,  $J_{1,2}$  = 1.0 Hz, H-1<sub>C</sub>), 4.94 (m, 2H, H-1<sub>E</sub>, 1<sub>B</sub>), 4.86 (d, 1H, H-1<sub>B</sub>), 4.82 (d, 1H, H-1<sub>C</sub>), 4.72 (d, 1H, H-1<sub>D'</sub>), 4.70 (d, 1H, H-1<sub>A'</sub>), 4.90-4.20 (m, 36H, 16 OCH<sub>2</sub>Ph, H-2<sub>B</sub>, 2<sub>B'</sub>, 3<sub>D</sub>, 3<sub>D'</sub>), 4.00-2.90 (m, 45H, H-2<sub>D</sub>, 4<sub>D</sub>, 5<sub>D</sub>, 6<sub>aD</sub>, 6<sub>bD</sub>, 3<sub>C</sub>, 4<sub>C</sub>, 5<sub>C</sub>, 2<sub>E</sub>, 3<sub>E</sub>, 4<sub>E</sub>, 5<sub>E</sub>, 6<sub>aE</sub>, 6<sub>bE</sub>, 3<sub>B</sub>, 4<sub>B</sub>, 5<sub>B</sub>, 2<sub>A</sub>, 3<sub>A</sub>, 4<sub>A</sub>, 5<sub>A</sub>, 2<sub>D'</sub>, 4<sub>D'</sub>, 5<sub>D'</sub>, 6<sub>aD'</sub>, 6<sub>bD'</sub>, 3<sub>C'</sub>, 4<sub>C'</sub>, 5<sub>C'</sub>, 2<sub>E'</sub>, 3<sub>E'</sub>, 4<sub>E'</sub>, 5<sub>E'</sub>, 6<sub>aE'</sub>, 6<sub>bE'</sub>, 3<sub>B'</sub>, 4<sub>B'</sub>, 5<sub>B'</sub>, 3<sub>A'</sub>, 4<sub>A'</sub>, 5<sub>A'</sub>, OCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 2.00 (s, 3H, NHAc), 1.88 (s, 3H, OAc), 1.86 (s, 3H, NHAc), 1.40-0.82 (m, 30H, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>, C(CH<sub>3</sub>)<sub>2</sub>). <sup>13</sup>C NMR partial:  $\delta$  172.1, 171.4, 170.2, 166.2, 165.9 (5C, C=O), 102.7 (C-1<sub>D'</sub>), 101.6, 101.2 (2C, C-1<sub>B</sub>, 1<sub>B'</sub>), 101.1 (C-1<sub>A</sub>), 99.8 (C-1<sub>D</sub>), 99.7 (C-1<sub>C</sub>), 98.2 (2C, C-1<sub>E</sub>, 1<sub>A'</sub>), 97.2 (2C, C-1<sub>C</sub>, 1<sub>E</sub>), 63.3, 62.6 (2C, C-6<sub>E</sub>, 6<sub>E'</sub>), 60.0, 57.8 (2C, C-2<sub>D</sub>, 2<sub>D'</sub>), 51.0 (CH<sub>2</sub>N<sub>3</sub>), 29.5, 29.4 (2C, C(CH<sub>3</sub>)<sub>2</sub>), 24.0 (2C, NHAc), 21.3 (OAc), 19.6, 19.5 (2C, C(CH<sub>3</sub>)<sub>2</sub>), 19.1, 18.9, 18.8, 18.5, 18.2, 18.1 (6C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>). FAB-MS of C<sub>200</sub>H<sub>225</sub>N<sub>5</sub>O<sub>48</sub> (M, 3446.9), *m/z* 3489.5 ([M+Na]<sup>+</sup>).

**2-Azidoethyl (2-O-acetyl-3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-O-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-O-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (**619**).**

To a solution of **618** (630 mg, 181  $\mu$ mol) in DCM (12 mL) was added dropwise, at 0°C, a solution of TFA (2 mL) and water (2 mL). The mixture was stirred for 3 h at this temperature, then concentrated by coevaporation first with water, then with toluene. The residue was eluted from a column of silica gel with 1:1 toluene-EtOAc to give **619** as a white foam (460 mg, 75 %); [ $\alpha$ ]<sub>D</sub> +9° (*c* 1, CHCl<sub>3</sub>). FAB-MS of C<sub>194</sub>H<sub>217</sub>N<sub>5</sub>O<sub>48</sub> (M, 3386.8), *m/z* 3409.2 ([M+Na]<sup>+</sup>). Anal. Calcd for C<sub>194</sub>H<sub>217</sub>N<sub>5</sub>O<sub>48</sub>·H<sub>2</sub>O: C, 68.43; H, 6.45; N, 2.06. Found: C, 68.40; H, 7.02; N, 1.61.

**2-Aminoethyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (**603**).** A mixture of **619** (130 mg, 38  $\mu$ mol) in MeOH (4 mL) was treated by MeONa until pH 9. The mixture was stirred for 1 h at rt, then heated at 55°C overnight. After cooling to rt, IR 120 (H<sup>+</sup>) was added until neutral pH, and the solution was filtered and concentrated. The residue was eluted from a column of

silica gel with 25:1 to 20:1 DCM-MeOH to give an amorphous residue. A solution of this residue in EtOH (1.5 mL), EtOAc (150  $\mu$ L), 1M HCl (66  $\mu$ L, 2 eq) was hydrogenated in the presence of Pd/C (100 mg) for 72 h at rt. The mixture was filtered and concentrated into a residue which was eluted from a column of C-18 with water, liophilized to afford  
 5 amorphous **603** as a white foam (41 mg, 71 %);  $[\alpha]_D -7^\circ$  (c 1, water).  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ) partial:  $\delta$  4.90 (m, 2H,  $J_{1,2} = 3.5$  Hz, H-1<sub>E</sub>, 1<sub>E'</sub>), 4.82, 4.76, 4.72, 4.67, 4.52, 4.51 (6 bs, 6H, H-1<sub>A</sub>, 1<sub>B</sub>, 1<sub>C</sub>, 1<sub>A'</sub>, 1<sub>B'</sub>, 1<sub>C'</sub>), 4.41 (d, 1H,  $J_{1,2} = 8.6$  Hz, H-1<sub>D</sub>\*), 4.29 (d, 1H,  $J_{1,2} = 8.6$  Hz, H-1<sub>D'</sub>\*), 1.77 (s, 6H, NHAc), 1.15-0.96 (m, 18H, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>);  $^{13}\text{C}$  NMR partial ( $\text{D}_2\text{O}$ ):  $\delta$  174.8, 174.7 (2C, C=O), 102.6 (C-1<sub>D</sub>\*), 102.9, 101.8, 101.6, 101.4, 101.3 (6C, C-1<sub>A</sub>, 1<sub>B</sub>, 1<sub>C</sub>, 1<sub>A'</sub>, 1<sub>B'</sub>, 1<sub>C'</sub>), 100.8 (C-1<sub>D</sub>\*), 97.9 (2C, C-1<sub>E</sub>, 1<sub>E'</sub>), 56.0, 56.4 (2C, 2 C-6<sub>D</sub>, 6<sub>D'</sub>), 22.7, 22.6 (2C, NHAc), 18.2, 17.2, 17.0, 16.9 (6C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>). HRMS: calculated for  $\text{C}_{66}\text{H}_{113}\text{N}_5\text{O}_{45}+\text{Na}$ : 1690.6544. Found 1690.6537.

**2-Azidoethyl (2-acetamido-3-O-acetyl-2-deoxy-4,6-O-isopropylidene- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-O-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-(2-acetamido-2-deoxy-4,6-O-isopropylidene- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-O-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-O-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-4,6-O-isopropylidene- $\beta$ -D-glucopyranoside (**621**).** A mixture of donor **607** (835 mg, 0.44 mmol) and acceptor **617** (590 mg, 0.3 mmol), 4 Å molecular sieves and dry 1,2-DCE (12 mL), was stirred for 1 h, then cooled to  $-30^\circ\text{C}$ . Triflic acid (35  $\mu$ L) was added. The stirred mixture was allowed to reach  $5^\circ\text{C}$  in 2.5 h.  $\text{Et}_3\text{N}$  (150  $\mu$ L) was added, and the mixture was filtered. After evaporation, the residue was eluted from a column of silica gel with 1:2 Cyclohexane-EtOAc and 0.2 %  $\text{Et}_3\text{N}$  to give **621** as a white foam (990 mg, 90 %);  $[\alpha]_D +10^\circ$  (c 1,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) partial:  $\delta$  6.95-7.90 (m, 90H, Ph), 5.98 (d, 1H,  $J_{2,\text{NH}} = 6.9$  Hz,  $\text{NH}_\text{D}$ ), 5.60 (d, 1H,  $J_{2,\text{NH}} = 7.5$  Hz,  $\text{NH}_\text{D}$ ), 5.45 (d, 1H,  $J_{2,\text{NH}} = 8.5$  Hz,  $\text{NH}_\text{D}$ ), 5.22 (dd, 1H,  $J_{1,2} = 1.0$ ,  $J_{2,3} = 3.0$  Hz, H-2<sub>C</sub>), 5.13 (dd, 1H,  $J_{1,2} = 1.0$ ,  $J_{2,3} = 3.0$  Hz, H-2<sub>C</sub>), 5.08 (d, 1H,  $J_{1,2} = 8.3$  Hz, H-1<sub>D</sub>), 5.07 (bs, 1H, H-1<sub>A</sub>), 5.04 (bs, 1H, H-1<sub>A</sub>), 4.97 (d, 1H,  $J_{1,2} = 3.0$  Hz, H-1<sub>E</sub>), 4.94 (d, 1H,  $J_{1,2} = 3.0$  Hz, H-1<sub>E</sub>), 4.90 (bs, 1H, H-1<sub>B</sub>), 4.86 (bs, 1H, H-1<sub>B</sub>), 4.82 (bs, 1H, H-1<sub>C</sub>), 4.73 (d, 1H, H-1<sub>D</sub>), 4.70 (bs, 1H, H-1<sub>C</sub>), 4.43 (d, 1H, H-1<sub>D</sub>), 4.20-4.80 (m, 16H,  $\text{CH}_2\text{Ph}$ ), 2.00, 1.85, 1.58 (3s, 9H, NHAc), 1.95 (s, 3H, OAc), 1.37-0.85 (m, 36H, 3  $\text{C}(\text{CH}_3)_2$ , H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>);  $^{13}\text{C}$  NMR partial:  $\delta$  171.7, 170.8, 169.8, 165.8, 165.4 (6C, C=O), 139.0-127.7 (Ph), 103.9 (C-1<sub>D</sub>), 102.8 (C-1<sub>D</sub>), 101.5 (2C, C-1<sub>B</sub>), 101.3 (C-1<sub>A</sub>), 101.1 (C-1<sub>A</sub>), 100.0 (C-1<sub>D</sub>), 99.5, 99.3 (3C,  $\text{C}(\text{CH}_3)_2$ ), 98.3 (C-1<sub>E</sub>), 98.1 (2C, C-1<sub>C</sub>, 1<sub>E</sub>), 97.8 (C-1<sub>C</sub>), 82.0, 81.7, 81.6, 81.4, 80.3, 80.2, 80.1, 79.5, 79.2, 78.9, 78.7, 78.4, 78.1, 77.9, 77.8, 77.6, 76.0, 75.8, 75.3, 75.2, 74.7, 74.4, 74.1, 74.0, 73.6, 73.5, 73.4, 73.3, 73.0, 72.7, 71.9, 71.4, 70.9, 70.8, 69.1, 69.0, 68.9, 68.7, 68.6, 68.5, 68.1, 67.8, 67.7, 67.5, 62.6,

62.3, 62.1, 60.8, 59.9, 57.9, 55.0 (3C, C-2<sub>D</sub>, 2<sub>D'</sub>, 2<sub>D''</sub>), 51.1 (CH<sub>2</sub>N<sub>3</sub>), 29.5, 29.4, 29.3 (3C, C(CH<sub>3</sub>)<sub>2</sub>), 24.0, 23.9, 23.5 (3C, NHAc), 21.3 (OAc), 19.7, 19.6, 19.2 (3C, C(CH<sub>3</sub>)<sub>2</sub>), 18.9, 18.8, 18.6, 18.5, 18.2, 18.1 (6C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>). FAB-MS for C<sub>211</sub>H<sub>242</sub>N<sub>6</sub>O<sub>53</sub> (M, 3710.2) *m/z* 3733.3 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>211</sub>H<sub>242</sub>N<sub>6</sub>O<sub>53</sub>: C, 68.31; H, 6.57; N, 2.27. Found: C, 68.17; H, 6.74; N, 2.12.

2-Azidoethyl (2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (622). The undecasaccharide 621 (990 mg, 0.27 mmol) was dissolved in MeOH (30 mL). MeONa was added until pH 9. The mixture was stirred for 3 h, then treated by IR 120 (H<sup>+</sup>) until neutral pH. The solution was filtered, and concentrated. The residue was eluted from a column of silica gel with 1:1 toluene-EtOAc and 0.2 % of Et<sub>3</sub>N to give 622 as a white foam (900 mg, 91 %); [α]<sub>D</sub><sup>25</sup> +15° (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR partial: δ 6.95-8.00 (m, 90H, Ph), 6.19 (bs, 1H, NH<sub>D</sub><sup>\*</sup>), 5.96 (d, 1H, *J*<sub>2,NH</sub> = 6.8 Hz, NH<sub>D</sub><sup>\*</sup>), 5.57 (d, 1H, *J*<sub>2,NH</sub> = 6.8 Hz, NH<sub>D</sub><sup>\*</sup>), 5.22 (dd, 1H, H-2<sub>C</sub><sup>\*</sup>), 5.13 (dd, 1H, H-2<sub>C</sub><sup>\*</sup>), 5.10 (d, 1H, H-1<sub>D</sub>), 5.07 (bs, 1H, H-1<sub>A</sub><sup>\*</sup>), 5.04 (bs, 1H, H-1<sub>A</sub><sup>\*</sup>), 4.96 (d, 1H, H-1<sub>E</sub><sup>\*</sup>), 4.94 (d, 1H, H-1<sub>E</sub><sup>\*</sup>), 4.85 (bs, 1H, H-1<sub>B</sub><sup>\*</sup>), 4.84 (bs, 1H, H-1<sub>B</sub><sup>\*</sup>), 4.82 (bs, 1H, H-1<sub>C</sub><sup>\*</sup>), 4.70 (d, 1H, H-1<sub>C</sub><sup>\*</sup>), 4.67 (d, 1H, H-1<sub>D</sub><sup>\*</sup>), 4.44 (d, 1H, H-1<sub>D</sub><sup>\*</sup>), 4.20-4.80 (m, 16H, CH<sub>2</sub>Ph), 2.00, 1.85, 1.58 (3s, 9H, NHAc), 1.37-0.80 (m, 36H, C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>). <sup>13</sup>C NMR partial: δ 172.8, 170.9, 170.3, 165.1, 164.7 (5C, C=O), 139.0-127.7 (Ph), 103.5, 103.1 (2C, C-1<sub>D</sub>, 1<sub>D'</sub>), 101.5 (2C, C-1<sub>B</sub>, 1<sub>B'</sub>), 101.2, 101.1 (2C, C-1<sub>A</sub>, 1<sub>A'</sub>), 99.9 (C-1<sub>D</sub><sup>\*</sup>), 99.0, 98.8, 98.7 (3C, C(CH<sub>3</sub>)<sub>2</sub>), 98.3 (C-1<sub>E</sub><sup>\*</sup>), 98.1 (2C, C-1<sub>C</sub><sup>\*</sup>, 1<sub>E</sub><sup>\*</sup>), 97.8 (C-1<sub>C</sub><sup>\*</sup>), 82.1, 82.0, 81.9, 81.7, 81.6, 81.5, 80.6, 80.3, 80.2, 80.1, 79.7, 79.1, 78.9, 78.5, 77.9, 77.6, 75.7, 74.9, 74.6, 74.3, 73.3, 73.0, 72.7, 71.9, 71.8, 69.1, 68.9, 68.7, 68.5, 68.0, 67.8, 67.7, 67.6, 67.5, 62.6, 62.3, 61.9, 60.5, 59.9, 57.4, 55.0 (3C, C-2<sub>D</sub>, 2<sub>D'</sub>, 2<sub>D''</sub>), 51.0 (CH<sub>2</sub>N<sub>3</sub>), 29.5, 29.3 (3C, C(CH<sub>3</sub>)<sub>2</sub>), 24.0, 23.9, 22.7 (3C, NHAc), 19.7, 19.6, 19.3 (3C, C(CH<sub>3</sub>)<sub>2</sub>), 19.0, 18.9, 18.6, 18.5, 18.2, 18.1 (6C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>). FAB-MS for C<sub>209</sub>H<sub>240</sub>N<sub>6</sub>O<sub>52</sub> (M, 3668.1) *m/z* 3690.8 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>211</sub>H<sub>242</sub>N<sub>6</sub>O<sub>53</sub>: C, 68.43; H, 6.59; N, 2.29. Found: C, 68.28; H, 6.72; N, 2.11.

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-

tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-

5 rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene- $\beta$ -D-glucopyranoside (623). A mixture of donor 606 (377 mg, 0.230 mmol) and acceptor 622 (427 mg, 0.115 mmol), 4 Å molecular sieves and dry 1,2-DCE (10 mL), was stirred for 1 h then cooled to -30°C. Triflic acid (20  $\mu$ L) was added. The stirred mixture was allowed to reach 5°C in 2.5 h. Et<sub>3</sub>N (150  $\mu$ L) was added, and the mixture filtered. After evaporation,  
10 the residue was eluted from a column of silica gel with 3:1 toluene-EtOAc and 0.2 % Et<sub>3</sub>N to give 623 as a foam (490 mg, 82 %); [ $\alpha$ ]<sub>D</sub> +20° (c 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR partial:  $\delta$  6.90-8.00 (m, 135H, Ph), 5.95 (d, 1H,  $J_{2,NH}$  = 6.6 Hz, NH<sub>D</sub>\*), 5.60 (d, 1H,  $J_{2,NH}$  = 8.0 Hz, NH<sub>D</sub>\*), 5.59 (d, 1H,  $J_{2,NH}$  = 7.5 Hz, NH<sub>D</sub>\*), 5.44 (dd, 1H, H-2<sub>C</sub>), 5.22 (dd, 1H, H-2<sub>C</sub>), 5.10 (dd, 1H, H-2<sub>C</sub>), 2.20 (s, 3H, OAc), 2.00, 1.85, 1.84 (3s, 9H, AcNH), 1.40-0.80 (m,  
15 45H, 3 C(CH<sub>3</sub>)<sub>2</sub>, H-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>, 6<sub>A''</sub>, 6<sub>B''</sub>, 6<sub>C''</sub>); <sup>13</sup>C NMR partial:  $\delta$  173.2, 172.6, 172.5, 171.3, 167.4, 167.0, 166.9 (C=O), 140.2-126.8 (Ph), 102.8, 102.7, 101.5, 101.3, 101.1, 99.9, 99.8, 98.1, 97.8, 82.0, 81.7, 81.5, 81.4, 80.2, 80.1, 79.6, 79.4, 78.9, 78.6, 78.0, 77.9, 77.6, 75.5, 73.4, 73.3, 73.0, 72.8, 71.9, 71.6, 69.4, 69.1, 69.0, 68.6, 67.8, 67.7, 67.6, 67.5, 62.6, 62.3, 60.0, 57.9, 57.7, 51.0 (CH<sub>2</sub>N<sub>3</sub>), 30.5 (3C, C(CH<sub>3</sub>)<sub>2</sub>), 25.0, 22.4 (3C, NHAc),  
20 22.9 (OAc), 20.7, 20.6, 20.2 (3C, C(CH<sub>3</sub>)<sub>2</sub>), 20.0, 19.9, 19.8, 19.7, 19.6, 19.3, 19.2, 19.1 (9C, C-6<sub>A</sub>, 6<sub>B</sub>, 6<sub>C</sub>, 6<sub>A'</sub>, 6<sub>B'</sub>, 6<sub>C'</sub>, 6<sub>A''</sub>, 6<sub>B''</sub>, 6<sub>C''</sub>). FAB-MS for C<sub>298</sub>H<sub>334</sub>N<sub>6</sub>O<sub>71</sub> (M, 5135.8)  $m/z$  5159.3 [M + Na]<sup>+</sup>. Anal. Calcd. for C<sub>298</sub>H<sub>334</sub>N<sub>6</sub>O<sub>71</sub>: C, 69.69; H, 6.55; N, 1.64. Found: C, 69.74; H, 6.72; N, 1.49.

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1  
25  $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)-  
30 (3,4-di-*O*-benzyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]-(2-*O*-benzoyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (624). To a solution of the pentadecasaccharide 623 (480 mg, 93  $\mu$ mol) in DCM (14 mL) was added dropwise at 0°C, a solution of 50% aq TFA (3.0 mL). The mixture was stirred for 3 h then concentrated by coevaporation first with water,  
35 then with toluene. The residue was eluted from a column of silica gel with 1:1 toluene-EtOAc to give 624 as a white foam (390 mg, 83 %); [ $\alpha$ ]<sub>D</sub> +12° (c 1, CHCl<sub>3</sub>); FAB-MS for C<sub>289</sub>H<sub>322</sub>N<sub>6</sub>O<sub>71</sub> (M, 5015.6)  $m/z$  5037.2 [M + Na]<sup>+</sup>.

**2-Aminoethyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (604).** A solution of the partially deprotected pentadecasaccharide **624** (390 mg, 77  $\mu$ mol) in MeOH (10 mL) was treated by MeONa until pH 10. The mixture was stirred overnight at 55°C. After cooling at rt, IR 120 ( $H^+$ ) was added until neutral pH. The solution was filtered, concentrated, and the residue was eluted from a column of silica gel with 20:1 DCM-MeOH to give the benzylated residue (252 mg). A solution of this residue in EtOH (3 mL), EtOAc (250  $\mu$ L) and 1M HCl (106  $\mu$ L) was hydrogenated in the presence of Pd/C (300 mg) for 48 h at rt. The mixture was filtered and concentrated, and the residue was eluted from a column of C-18 with water/ $CH_3CN$ , and freeze-dried to afford amorphous **604** (127 mg, 65 %);  $[\alpha]_D -5^\circ$  ( $c$  1, water).  $^1H$  NMR ( $D_2O$ ) partial:  $\delta$  5.13 (m, 3H, H-1 $_E$ , 1 $_E'$ , 1 $_E''$ ), 5.07, 4.99, 4.95, 4.90, 4.75 (m, 9H, H-1 $_A$ , 1 $_B$ , 1 $_C$ , 1 $_A'$ , 1 $_B'$ , 1 $_C'$ , 1 $_A''$ , 1 $_B''$ , 1 $_C''$ ), 4.63, 4.51 (2d, 3H,  $J_{1,2} = 8.5$  Hz, H-1 $_D$ , 1 $_D'$ , 1 $_D''$ ), 2.00 (s, 9H, NHAc), 1.30-1.18 (m, 27H, H-6 $_A$ , 6 $_B$ , 6 $_C$ , 6 $_A'$ , 6 $_B'$ , 6 $_C'$ , 6 $_A''$ , 6 $_B''$ , 6 $_C''$ );  $^{13}C$  NMR ( $D_2O$ ) partial:  $\delta$  174.8, 174.7 (3C, C=O), 102.9, 102.6, 101.7, 101.3, 100.8, 97.9, 81.8, 81.7, 79.6, 79.0, 76.3, 76.2, 73.0, 72.7, 72.4, 72.1, 71.6, 70.5, 70.1, 70.0, 69.7, 69.6, 69.4, 68.7, 68.6, 66.0, 61.0, 56.0, 55.4, 39.8, 22.7, 22.6 (NHAc), 18.2, 17.2, 17.0, 16.9 (9C, C-6 $_A$ , 6 $_B$ , 6 $_C$ , 6 $_A'$ , 6 $_B'$ , 6 $_C'$ , 6 $_A''$ , 6 $_B''$ , 6 $_C''$ ). MALDI-MS for  $C_{98}H_{166}N_4O_{67}Na$  (M, 2493.96)  $m/z$  2494.96.

(*S*-Acetylthiomethyl)carbonylaminoethyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (**620**). A solution of SAMA-Pfp (2.8 mg, 9.5  $\mu$ mol) in CH<sub>3</sub>CN (60  $\mu$ L) was added to the aminoethyl deca-saccharide **603** (6.4 mg, 3.84  $\mu$ mol) in 0.1M phosphate buffer (pH 7.4, 500  $\mu$ L). The mixture was stirred at rt for 1 h and purified by RP-HPLC to give **620** (4.2 mg, 61%). HPLC (230 nm): Rt 14.17 min (99.9% pure, Kromasil 5  $\mu$ m C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01 M aq TFA at 1 mL/min flow rate). ES-MS for C<sub>70</sub>H<sub>117</sub>N<sub>3</sub>O<sub>47</sub>S (M, 1784.76) *m/z* 1784.70.

(S-Acetylthiomethyl)carbonylaminoethyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -



-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (625). A solution of SAMA-Pfp (2.8 mg, 9.6 μmol) in CH<sub>3</sub>CN (50 μL) was added to the pentadecasaccharide 604 (9.4 mg, 3.8 μmol) in 0.1M phosphate buffer (pH 7.4, 500 μL). The mixture was stirred at rt for 2 h and  
 5 purified by RP-HPLC to give 625 (6.3 mg, 63%). HPLC (230 nm): Rt 13.97 min (99.0% pure, Kromasil 5 μm C18 100 Å 4.6x250 mm analytical column, using a 0-20% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate. ES-MS for C<sub>102</sub>H<sub>170</sub>N<sub>4</sub>O<sub>69</sub>S (M, 2588.53) *m/z* 2588.67.

**PADRE (thiomethyl)carbonylaminoethyl α-L-rhamnopyranosyl-**  
 10 **(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-**  
**rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-**  
**rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-**  
**α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (601).**  
 Compound 620 (6.0 mg, 3.36 μmol) was dissolved in water (300 μL) and added to a  
 15 solution of PADRE-Mal (7.1 mg, 4.0 μmol) in a mixture of water (630 μL), CH<sub>3</sub>CN (120 μL) and 0.1M phosphate buffer (pH 5.6, 750 μL). 68 μL of a solution of hydroxylamine hydrochloride (139 mg/mL) in 0.1M phosphate buffer (pH 5.6) was added and the mixture was stirred for 2 h. RP-HPLC purification gave the pure target 601 (5.2 mg, 44%). HPLC (230 nm): Rt 10.03 min (100% pure, Kromasil 5 μm C18 100 Å 4.6x250 mm analytical  
 20 column, using a 20-50% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate). ES-MS Calcd for C<sub>153</sub>H<sub>254</sub>N<sub>24</sub>O<sub>65</sub>S (M, 3501.91) *m/z* 3501.15.

**PADRE (thiomethyl)carbonylaminoethyl α-L-rhamnopyranosyl-(1→**  
**2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-**  
**(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→**  
 25 **2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-**  
**(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→**  
**2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-**  
**(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (602).** Compound 625 (10.3 mg, 3.98 μmol) was dissolved in water (350 μL) and added to a solution of PADRE-Mal (9.0 mg, 5.0 μmol) in a mixture of water (740 μL), CH<sub>3</sub>CN (140 μL) and 0.5M phosphate buffer (pH 5.6, 890 μL). 80 μL of a solution of hydroxylamine hydrochloride (139 mg/mL) in 0.5M phosphate buffer (pH 5.7) was added, and the mixture was stirred for 3 h. RP-HPLC purification gave the pure conjugate 602 (11.5 mg, 67%). HPLC (230 nm): Rt 9.07 min (100% pure, Kromasil 5 μm C18 100 Å 4.6x250 mm analytical column, using a 20-50%  
 30 linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate). ES-MS Calcd for C<sub>185</sub>H<sub>307</sub>N<sub>25</sub>O<sub>87</sub>S (M, 4305.69) *m/z* 4305.45.

**G. Synthesis of biotinylated analogues of oligosaccharides representative of fragments of the O-SP of *Shigella flexneri* 2a**

**(+)-Biotinyl-3,6-dioxaoctainediaminyl-**

**(thiomethyl)carbonylaminoethyl  $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (708).** Compound **701** (5.0 mg, 7.26  $\mu$ mol) was dissolved in water (280  $\mu$ L) and added to a solution of **707** (3.2 mg, 7.26  $\mu$ mol) in 0.5 M phosphate buffer (pH 6.0, 400  $\mu$ L). A 2 M solution of hydroxylamine in 0.5 M phosphate buffer (150  $\mu$ L) was added and the mixture was stirred at rt for 1 h. More **707** (1.5 mg, 2.85  $\mu$ mol) in 0.5 M phosphate buffer (300  $\mu$ L) was added, and the mixture was stirred for 1h30 at rt. RP-HPLC purification gave the pure neoglycopeptide **708** (5.7 mg, 67%). ES-MS for C<sub>47</sub>H<sub>77</sub>N<sub>7</sub>O<sub>23</sub>S<sub>2</sub> (M, 1171.5) *m/z* 1171.45.

**(+)-Biotinyl-3,6-dioxaoctainediaminyl-**

**(thiomethyl)carbonylaminoethyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (709).** Compound **702** (10.0 mg, 12.0  $\mu$ mol) was dissolved in water (500  $\mu$ L) and added to a solution of **707** (12.6 mg, 20.0  $\mu$ mol) in 0.5 M phosphate buffer (pH 6, 220  $\mu$ L). A 2 M solution of hydroxylamine in 0.5 M phosphate buffer (300  $\mu$ L) was added and the mixture was stirred at rt for 2 h. Since HPLC control showed that some **702** remained, the pH of the mixture was adjusted to 5 by dropwise addition of diluted aq NH<sub>3</sub>, and the mixture was stirred for 1 h more at rt. RP-HPLC purification gave the pure neoglycopeptide **709** (12.6 mg, 80%). ES-MS Calcd for C<sub>109</sub>H<sub>181</sub>N<sub>23</sub>O<sub>35</sub>S<sub>2</sub> (M, 2405.85) *m/z* 1317.51.

**(+)-Biotinyl-3,6-dioxaoctainediaminyl-**

**(thiomethyl)carbonylaminoethyl  $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (710).** Compound **703** (3.8 mg, 3.87  $\mu$ mol) was dissolved in water (250  $\mu$ L) and added to a solution of **707** (3 mg, 5.7  $\mu$ mol) in 0.5 M phosphate buffer (pH 5.8, 250  $\mu$ L). A 2 M solution of hydroxylamine in 0.5 M phosphate buffer (75  $\mu$ L) was added and the mixture was stirred at rt for 1 h. RP-HPLC purification gave the pure neoglycopeptide **710** (4.6 mg, 81%). ES-MS Calcd for C<sub>59</sub>H<sub>97</sub>N<sub>7</sub>O<sub>31</sub>S<sub>2</sub> (M, 1464.6) *m/z* 1463.57.

**(+)-Biotinyl-3,6-dioxaoctainediaminyl-**

**(thiomethyl)carbonylaminoethyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-[ $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)-2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (711).** Compound **704** (2.5 mg, 2.11  $\mu$ mol) was dissolved in water (85  $\mu$ L) and added to a solution of **707** (1.7 mg, 3.2  $\mu$ mol) in 0.5 M phosphate buffer (pH 5.9, 215  $\mu$ L). A 2 M solution of hydroxylamine in 0.5 M phosphate buffer (45  $\mu$ L) was added and the mixture was stirred at rt for 2 h. RP-HPLC purification gave the pure neoglycopeptide **711** (2.5 mg, 71%). HPLC (230 nm): Rt 17.03 min (100% pure, Kromasil 5  $\mu$ m C18 100 Å 4.6x250 mm

analytical column, using a 0-30% linear gradient over 20 min of CH<sub>3</sub>CN in 0.01M aq TFA at 1 mL/min flow rate). ES-MS for C<sub>67</sub>H<sub>110</sub>N<sub>8</sub>O<sub>36</sub>S<sub>2</sub> (M, 1667.78) *m/z* 1667.45.

**(+)-Biotinyl-3,6-dioxaoctainediaminyl-**

**(thiomethyl)carbonylaminoethyl** **α-L-rhamnopyranosyl-(1→2)-α-L-**

- 5 **rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-**  
**2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-**  
**rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-**  
**2-acetamido-2-deoxy-β-D-glucopyranoside (712).** Compound 705 (4.0 mg, 2.24 μmol)  
 10 was dissolved in water (85 μL) and added to a solution of 707 (1.8 mg, 3.3 μmol) in 0.5 M  
 phosphate buffer (pH 5.9, 220 μL). A 2 M solution of hydroxylamine in 0.5 M phosphate  
 buffer (45 μL) was added and the mixture was stirred at rt for 2 h. RP-HPLC purification  
 gave the pure neoglycopeptide 712 (4.5 mg, 89%). HPLC (230 nm): Rt 16.69 min (100%  
 pure, Kromasil 5 μm C18 100 Å 4.6x250 mm analytical column, using a 0-30% linear  
 gradient over 20 min of CH<sub>3</sub>CN in 0.01 M aq TFA at 1 mL/min flow rate). ES-MS for  
 15 C<sub>91</sub>H<sub>115</sub>N<sub>8</sub>O<sub>53</sub>S<sub>2</sub> (M, 2268.35) *m/z* 2267.72.

**(+)-Biotinyl-3,6-dioxaoctainediaminyl-**

**(thiomethyl)carbonylaminoethyl** **α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-**  
**(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-**  
**deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-**

- 20 **rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-**  
**2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-**  
**rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-**  
**2-acetamido-2-deoxy-β-D-glucopyranoside (713).** Compound 706 (5.7 mg, 2.21 μmol)  
 25 was dissolved in water (85 μL) and added to a solution of 707 (1.7 mg, 3.2 μmol) in 0.5 M  
 phosphate buffer (pH 5.9, 220 μL). A 2 M solution of hydroxylamine in 0.5 M phosphate  
 buffer (45 μL) was added and the mixture was stirred at rt for 2 h. RP-HPLC purification  
 gave the pure neoglycopeptide 713 (4.8 mg, 71%). HPLC (230 nm): Rt 16.35 min (100%  
 pure, Kromasil 5 μm C18 100 Å 4.6x250 mm analytical column, using a 0-30% linear  
 gradient over 20 min of CH<sub>3</sub>CN in 0.01 M aq TFA at 1 mL/min flow rate). ES-MS for  
 30 C<sub>123</sub>H<sub>203</sub>N<sub>9</sub>O<sub>75</sub>S<sub>2</sub> (M, 3072.13) *m/z* 3072.17.

**II-The serum immunoglobulin G-mediated response to serotype-specific determinants of *Shigella flexneri* lipopolysaccharide protects against experimental shigellosis**

- Both intestinal secretory IgA (SIgA) and serum IgG specific for the O-  
 35 antigen (O-SP, figure 29), the polysaccharide part of the bacterial lipopolysaccharide  
 (LPS) are elicited upon *Shigella* infection, the causative agent of bacillary dysentery.  
 However, the respective protective roles of local and systemic humoral immunity remain  
 unclear

The ineffectiveness of parenterally injected inactivated whole-cell vaccines in inducing protection, despite the high level of anti-LPS serum IgG antibodies raised, has led to the belief that serum antibodies do not confer protection (Formal et al., ProC; Soc. Exp. Biol. Med., 1967, 125, 347-; Higgins et al., Am. J. Trop. Med., 1955, 4, 281-288). However, several indirect pieces of evidence suggest that anti-O-SP serum IgG may confer protection during natural infection. A correlation was found between the level of anti-LPS IgG antibodies and resistance to shigellosis among Israeli soldiers (Cohen et al., J. Inf. Dis., 1988, 157, 1068; Cohe, et al., J. Clin. Microbial., 1991, 29,386), and an inverse relationship exists between the age of incidence of shigellosis and the presence of IgG antibodies to *Shigella* LPS (Passwell et al., Pediatr. Infect. Dis., 1995, 14, 859-; Van de Verg et al., J. Infect. Dis., 1992, 166, 158-161). In addition, a detoxified LPS-based conjugate vaccine administered parenterally and eliciting mainly, if not only, serum antibodies has been shown to induce protective immunity (Cohen et al., lancet, 1997, 349, 155-).

In the current study, using the mouse model of pulmonary infection and specific polyclonal serum or monoclonal IgG, the protective role of serum IgG recognizing serotype-specific LPS determinants or peptide epitopes on the invasins IpaB and IpaC was addressed.

#### **A) Materials and Methods**

##### **1) Bacterial strains**

M90T, an invasive isolate of *S. flexneri* serotype 5a, and 454, an invasive isolate of *S. flexneri* serotype 2a, were the virulent strains of reference. For i.n. infection, bacteria were routinely grown on Luria Bertoni agar plates at 37°C. They were recovered from plates and bacterial dilutions were performed in 0.9% NaCl with the consideration that, for an optical density of 1 at 600 nm, the bacterial concentration was  $5 \times 10^8$  colony forming units (c.f.u)/ml. Killed bacteria for systemic immunizations were prepared from bacterial cultures at stationary phase, diluted to  $5 \times 10^8$  c.f.u. /ml in 0.9% NaCl, and then incubated at 100°C for 1h. They were then kept at -20°C in aliquots.

##### **2) Production and characterization of mAbs specific for *S. flexneri* serotype 2a and 5a LPS**

BALB/c mice were immunized intraperitoneally (i.p.) with  $10^7$  c.f.u. of killed *S. flexneri* 5a or *S. flexneri* 2a bacteria three times at 3 week-intervals. Mice eliciting the highest anti-LPS antibody response were given an intravenous booster injection 3 days before being sacrificed for splenic B cell fusion according to Kohler and Milstein (Eur. J. Immunol., 1976, 6, 511-519). Hybridoma culture supernatants were screened for antibody production by ELISA using LPS purified from *S. flexneri* X, Y, 5a, 5b, 2a, 2b, 1a and 3a, respectively. The hybridoma cells secreting murine IgG (mIgG) reacting specifically with LPS homologous to the strain used for immunization, *i. e.* recognizing serotype-specific

determinants on the LPS O-SP, were selected. A panel of mIgG representative of the four murine IgG subclasses was used for the study. Those selected were then cloned by limiting dilution, and injected i. p. into histocompatible mice for ascitis production. mIgG were precipitated with 50% ammonium sulfate from ascitis fluid, centrifuged, and dialysed against PBS before being purified using ion-exchange chromatography as previously described (Barzu et al., Infect. Immun., 1998, 65, 1599-1605; Phalipon et al., Infect. Immun., 1992, 60, 1919-1926). The avidity of anti-LPS mIgG for LPS was determined as follows: various concentrations of LPS were incubated in solution overnight at 4°C with a defined amount of a given mIgG until equilibrium was reached. Each mixture was then transferred to a microtiter plate previously coated with homologous purified LPS. Bound antibodies were detected by using peroxidase-conjugated anti-mouse immunoglobulins specific for IgG subclasses. IC<sub>50</sub> was defined as the concentration of LPS required to inhibit 50% of mIgG binding to LPS.

### 3) ELISA

Hybridoma culture supernatants were tested by ELISA for the presence of anti-LPS antibodies as previously described (Barzu et al., Infect. Immun., 1993, 61, 3825-3831; Phalipon et al., Infect. Immun., 1992, 60, 1919-1926) except that LPS purified according to Westphal (Methods Carbohydr. Chem., 1965, 5, 83-91) was used at a concentration of 5 µg/ml in PBS. As secondary antibodies, anti-mouse IgG- or IgM- or IgA-alkaline phosphatase-labeled conjugate (SIGMA) were used at a dilution of 1:5,000. To measure the anti-LPS antibody titer in polyclonal serum, biotin-labeled Abs to IgG and its different subclasses (IgG1, -2a, -2b, -3) (PHARMINGEN) and avidin conjugated with alkaline phosphatase (SIGMA) were used at a dilution of 1:5,000. Antibody titers were defined as the last dilution of the sample giving an OD at least twice that of the control.

### 4) Active and passive immunization of mice

To obtain polyclonal serum, mice were immunized i.p. with  $5 \times 10^7$  killed bacteria, three times at 3 week-intervals. After bleeding, anti-LPS antibody titer in the polyclonal sera was measured by ELISA, as described above, and those ranging from low (1/4,000) to high titer (1/64,000) were used for i.n. passive transfer. Purified mAbs (20 or 2 µg) were also administered intranasally. All i.n. administrations were performed using a volume of 20 µl and mice previously anesthetized via the intramuscular route with 50 µl of a mixture of 12.5% ketamine (MERIAL) and 12.5% acepromazine (VETOQUINOL). Each experiment was performed using 10 mice per group and was repeated three times.

### 5) Protection experiments

The protective capacity of the antibodies was analysed using the murine model of pulmonary infection previously described (Voino et al., Acta Morpho., 1961, XI, 440-; Phalipon et al., J. exp. Med., 1995, 182, 769-). Intranasal challenge was performed using either  $10^9$  live virulent bacteria when protection was assessed by mortality assay or

10<sup>8</sup> bacteria when protection was assessed by measurement of the lung-bacterial load. Naive mice were used as controls in each experiment. Mice immunized i.p. were challenged i.n. with virulent bacteria, 3 weeks after the last immunization. Mice passively transferred i.n. with polyclonal sera or with purified mAbs were challenged 1h after administration of the mAbs. Measurement of lung-bacterial load was performed at 24h post infection as follows. Mice were sacrificed by cervical dislocation and lungs were removed « *en bloc* » and ground in 10 ml sterile PBS (Ultra Turrax T25 apparatus, Janke and Kunkel IKA Labortechnik GmbH). Dilutions were then plated on Trypticase Soy Broth plates for c.f.u. enumeration. Each experiment was performed using 10 mice per group and was repeated three times.

#### 5) Histopathological studies

Mice were anesthetized, their trachea catheterized, and 4% formalin injected in order to fill the bronchoalveolar space. Lungs were then removed and fixed in 4% formalin before being processed for histopathological studies. Ten-micrometer paraffin sections were stained with Hematoxiline and Eosin (HE), and observed with a BX50 Olympus microscope (Olympus Optical, Europa, GmbH).

#### 6) Statistical analysis

Significant differences were compared using the Student's test. Probability values < 0.05 were considered significant.

### B) Results

1) Protection conferred upon systemic immunization or intranasal administration of specific immune serum.

In order to address the role of the systemic anti-LPS IgG antibody response in protection against the mucosal infection, the protection conferred against i.n. challenge with a lethal dose of *S. flexneri* 2a bacteria in mice immunized i.p. with the homologous killed bacteria was assessed. Antibodies induced upon such an immunization were mainly anti-LPS IgG antibodies with all the IgG subclasses similarly elicited (Figure 30A). No mucosal response was elicited, as reflected by the absence of anti-LPS antibody response detectable in the bronchoalveolar lavage of immunized mice. Only 40% of the immunized mice survived the i.n. challenge, whereas 100% of naive mice succumbed. The low efficacy of systemic immunization in inducing protection could be due to either the inability of anti-LPS IgG to be protective or the absence of the protective antibodies (or their presence but in insufficient amount) in the mucosal compartment at the time of i.n. challenge.

Therefore, it was tested whether the anti-LPS IgG antibodies may confer protection if present locally prior to mucosal challenge. Polyclonal sera exhibiting different anti-LPS antibody titers were intranasally administered to naive mice 1h prior to i.n. infection with a sublethal dose of *S. flexneri* 2a bacteria. Protection was assessed by the

reduction of the lung-bacterial load in comparison to control mice and mice receiving preimmune serum. In contrast to control mice and mice receiving preimmune serum, naive mice receiving anti-LPS IgG serum showed a significant decrease of the lung-bacterial load. The reduction was dependent on the amount of anti-LPS IgG antibodies administered as reflected by the anti-LPS antibody titer of the immune serum used for passive transfer. Thus, the highest reduction was obtained with serum having the highest anti-LPS antibody titer (1/64,000) (Figure 30B, c);  $p=5 \times 10^{-6}$  in comparison to mice receiving preimmune serum). However, in mice receiving immune serum with lower anti-LPS antibody titer (1/16,000 and 1/4,000) (Figure 30B, a and b), even if less efficient, the decrease of the bacterial load was still significant in comparison to mice receiving preimmune serum ( $p = 0,027$  and  $0,015$ , respectively).

These results demonstrated that, if present locally at the time of mucosal challenge, the anti-LPS IgG antibodies were protective, thus limiting bacterial invasion.

2) Protective capacity of different subclasses of mIgG specific for *S. flexneri* 2a LPS

Depending of the infecting strain, different subclasses of IgG specific for LPS are induced following natural *Shigella* infection (Islam et al., Infect. Immun., 1995, 63, 2045-2061). To test whether all subclasses exhibit similar protective capacity, murine mIgG specific for serotype determinants on the O-SP and, representative of each of the four murine IgG subclasses were obtained. Upon screening of hybridomas for their reactivity with LPS from *S. flexneri* serotype X, Y, 5a, 5b, 2a, 2b, 1a, 3a, respectively, five mIgG specific for *S. flexneri* 2a LPS were selected : mIgG F22-4 (IgG1), mIgG D15-7 (IgG1), mIgG A2-1 (IgG2a), mIgG E4-1 (IgG2b) and mIgG C1-7 (IgG3). These hybridomas have been deposited on april 20, 2004, at the "Collection National de Culture des Microorganismes" from INSTITUT PASTEUR, 25 rue du Docteur Roux, 75724 PARIS CEDEX 15, FRANCE, under the registration number I-3197, I-3198, I-3199, I-3200 and I-3201, for A2-1, C1-7, D15-7, E4-1 and F22-4, respectively.

The avidity of each mIgG for LPS, defined by  $IC_{50}$ , ranged from 2 to 20 ng/ml. To analyse the protective capacity of the selected mAbs, naive mice were administered i.n. with each of the purified mIgG prior to i.n. challenge with a *S. flexneri* sublethal dose. Upon challenge, lung-bacterial load in mice passively administered with 20  $\mu$ g of each of the mIgG specific for *S. flexneri* 2a LPS was significantly reduced in comparison to mice receiving PBS (Figure 31A). Upon passive transfer using 2  $\mu$ g of mIgG, only mIgG D15-7, A2-1 and E4-1 were shown to significantly reduce the lung-bacterial load in comparison to control mice, but with much less efficiency than that observed using 20  $\mu$ g (Fig. 31A). As shown in Figure 31B, reduction of lung-bacterial load in mice receiving 20  $\mu$ g of mIgG was accompanied by a reduction of inflammation and therefore of subsequent tissue destruction. In comparison to control mice showing an acute

broncho-alveolitis with diffuse and intense polymorphonuclear cell infiltration (Figure 31B, *a, b*) associated with tissular dissemination of bacteria (Figure 2B, *c*), only restricted areas of inflammation were observed in antibody-treated mice, essentially at the intra- and peribronchial level (Figure 31B, *d, e*), where bacteria localized (Figure 31B, *f*). Following passive administration with 2  $\mu$ g of mIgG, inflammation resembled that of the control mice with a similar pattern of Polymorphonuclear (PMN) infiltration and tissue destruction, in accordance with the very low, if any, reduction in lung-bacterial load.

These results with murine monoclonal antibodies (mAbs) of the C isotype (mIgG) representative of the different IgG subclasses and specific for serotype-specific determinants on the O-SP, demonstrated that each IgG subclass exhibited a similar serotype-specific protective capacity, with significant reduction of the lung-bacterial load and of subsequent inflammation and tissue destruction. These antibodies may confer protection by different pathways involving or not the complement cascade. In the present study, all the different murine IgG subclasses were shown to be protective, suggesting that depending on the subclass, different mechanisms may be involved in IgG-mediated protection. Whereas antibody-dependant cellular cytotoxicity (ADCC) has been reported for *Shigella*-specific secretory IgA and lymphocytes from the gut-associated lymphoid tissues (Tagliabue et al., Nature, 1983, 306, 184-186), *Shigella* IgG-mediated ADCC occurs *in vitro* with splenic T cells but not with T lymphocytes from the GALT (Tagliabue et al., J. Immunol., Nature, 1984, 133, 988-992). Further studies using mice deficient for T cells or for proteins of the complement cascade will be required to analyze the IgG mediated protective mechanisms *in vivo*.

### 3) Serotype-specific protection induced by the anti-LPS mIgG

Antibodies specific for epitopes common to several serotypes of a given species as well as serotype-specific antibodies are elicited upon natural or experimental infection (Rasolofo-Razanamparany, Infect. Immun., 2001, 69, 5230-5234, Van de Verg et al., Vaccine, 1996, 14, 1062-1068). However, the serotype-specific protection observed following natural or experimental infection suggests that the antibodies directed against serotype determinants play a major protective role (Du Pont et al., J. Infect. Dis., 1972, 125, 12-; Mel et al., Bull. W.H.O., 1968, 39, 375-380). For instance, mIgA specific for *S. flexneri* serotype 5a has been shown to protect only against homologous challenge (Phalipon et al., J. Exp. Med., 1995, 182, 769-). Therefore, it was tested whether the protection observed with the anti-LPS mIgG obtained in this study was also serotype specific. Mice passively administered with 20  $\mu$ g of mIgG C1 specific for *S. flexneri* 2 were protected against homologous challenge, but not upon heterologous challenge with *S. flexneri* 5a bacteria (Fig. 32A). Similarly, mice receiving 20  $\mu$ g of mIgG C20, a mAb specific for *S. flexneri* serotype 5a and, of the same isotype than mIgG C1, *i.e.* IgG3 showed a significant reduction of lung-bacterial load upon i.n. challenge with *S. flexneri*



5a, but not with *S. flexneri* 2a (Figure 32A). In mice protected against homologous challenge, inflammation was dramatically reduced with a slight intra- and peribronchial PMN infiltrate remaining present (Figure 32B, *b* and *c*). In contrast, in mice not protected upon heterologous challenge (Figure 32B, *a* and *d*), inflammation and tissue destruction were similar to those observed in control mice (Figure 32B, and *b*).

The protective role of the serotype-specific antibody response has been firstly emphasized in a study using a monoclonal dimeric IgA (mIgA) specific for a *S. flexneri* serotype 5a determinant (Phalipon et al., J. Exp. Med., 1995, 182, 769-51). The results presented here demonstrate that mIgGs specific for *S. flexneri* serotype 2a or serotype 5a also confer serotype-specific protection. It seems that whatever the antibody isotype and the bacterial strain, the serotype-specific antibody response is protective against homologous bacterial challenge. It should be noted that using the same amount of mIgA and mIgG specific for *S. flexneri* 5a, both exhibiting a similar IC<sub>50</sub> for LPS, reduction in lung-bacterial load was much more efficient with mIgA. Actually, in contrast to mIgG, protection was observed in the presence of 2µg of mIgA. The discrepancy between the two isotypes may be due to the dimeric/polymeric (d/p) form of mIgA, which mimicks the IgA response at the mucosal surface. In contrast to monomeric IgG, interaction of d/p IgA exhibiting at least four antigen-binding sites with a specific determinant highly repeated on the bacterial O-SP surface may lead to the formation of aggregates that are efficiently removed by local physical mechanisms (Corthésy et al., Curr. Top. Microbiol. Immunol., 1999, 236, 93-111). Also, quantitative assessment of IgG and IgA subclass producing cells in the rectal mucosa during shigellosis in humans has revealed the predominance of the IgA response. The IgG response which is about 50 times lower than the IgA response is mainly IgG2 and correlates with the presence of specific IgG2 in serum. This correlation suggests that the majority of the *Shigella* specific serum antibodies are derived from the rectal mucosa (Islam et al., J. Clin. Pathol., 1997, 50, 513-520). Together, these results suggest that in the situation where both local and systemic anti-LPS antibody responses are induced, as for example upon natural infection, the local SIgA-mediated response will be the major protective response, with the IgG-mediated response possibly contributing to a lesser extent to local protection.

On the other hand, the data presented here suggest that in the absence of local SIgA-mediated response, as for example upon vaccination via the systemic route using glycoconjugate vaccines, the systemic anti-O-SP response induced is effective in protecting against homologous *Shigella* infection, if the effectors are present locally. Previous reports have shown that serum IgGs may protect from gastrointestinal infections (Bougoudogo et al., Bull. Inst. Pasteur, 1995, 93, 273-283; Pier et al., Infect. Immun., 1995, 63, 2818-2825). Therefore, it should be admitted that serum IgG efficiently gain access to the intestinal barrier in order to prevent bacterial invasion and dissemination.

How IgG crosses the epithelial barrier to function in mucosal immunity remains unclear. One possible pathway is passive transudation from serum to intestinal secretions (Batty et al., J. Pathol., Bacteriol., 1961, 81, 447-458; McCleery et al., Digestion, 1970, 3, 213-221; Wernet et al., J. Infect. Dis., 1971, 124, 223-226). After its passage of the intestinal barrier through M cells and its interaction with resident macrophages and epithelial cells, *Shigella* initiates an inflammatory response leading to infiltration of the infected tissues with polymorphonuclear cells (Philpott et al., Philos. Trans. R. Soc. Lond. B. Biol. Sci., 2000, 29, 575-586). It may therefore be reasonably envisioned that specific serum IgGs transudate to the intestinal tissue during this inflammatory process that occurs very soon after bacterial translocation. Another explanation could be the involvement of the FcRn receptor in IgG transport. FcRn was firstly identified as the Fc receptor responsible for transferring maternal IgGs from mother's milk across the intestinal EC of the neonatal gut of rodents. Much evidence supports the concept that FcRn is ubiquitously expressed in adult tissues and plays a role in IgG homeostasis, dealing with IgG half-life (Ghetie et al., Ann. Rev. Immunol., 2000, 18, 739-766). It has been recently reported that this receptor is expressed by enterocytes in human adults and mediates transcytosis of IgG in both direction across the intestinal epithelial monolayer (Ramaligan et al., EMBO J., 1997, 21, 590-601). Further investigation is required to improve the knowledge on the role played by FcRn in IgG-mediated protection of the intestinal barrier against enteropathogens. Nevertheless, the existence of such a pathway already enlarges the current view of the humoral response at mucosal surfaces.

#### 4) Absence of protection induced by the mIgG specific for *S. flexneri* invasins

The invasins IpaB and IpaC are essential to the expression of the *Shigella* invasive phenotype (Ménard et al., J. Bacteriol., 1993, 175, 5899-5906). Moreover, they are targets for the humoral response since antibodies specific for both proteins are detected in sera of patients convalescent from shigellosis (dam et al., J. Clin. Microbiol., 1993, 31, 454-457; Oaks et al., Infect. Immun., 1986, 53, 57-63; Oberhelman et al., Infect. Immun., 1991, 59, 2341-2350; Van de Verg et al., J. Infect. Dis., 1992, 166, 158-161). To assess whether the anti-invasin antibody response may contribute to protection, in addition to the anti-LPS antibody response, mIgG recognizing different epitopes on IpaB or IpaC, were used (Barzu et al., Infect. Immun., 1993, 61, 3825-3831; Phalipon et al., Infect. Immun., 1992, 60, 1919-1926). Whatever the dose used, in contrast to mIgG C20, no reduction in lung-bacterial load was measured upon challenge in mice treated with mIgG H16 and mIgG H4 recognizing distinct epitopes in the central region of IpaB or with mIgG J22 and mIgG K24 recognizing the N- and the C-termini domain of IpaC, respectively (Figure 33). Protection was also not observed upon combining anti-IpaB and anti-IpaC mIgG.

The results presented here demonstrated that mIgG specific for IpaB or IpaC are not protective despite the fact that they are directed against epitopes located in different regions of these proteins (Barzu et al., Infect. Immun., 1993, 61, 3825-3831; Phalipon et al., Infect. Immun., 1992, 60, 1919-1926) and that they have been shown to interfere with their functional properties in *in vitro* studies (Barzu et al., Infect. Immun., 1998, 65, 1599-1605; Ménard et al., Cell, 1994, 79, 515-525). The most likely explanation is that these invasins, that are secreted through the type III secretion apparatus, are injected straight into the host cell, upon contact of the bacterium with the cell membrane (Ménard et al., EMBO J., 1994, 13, 5293-5302; Blocker et al., Mol. Microbiol., 2001, 39, 652-663). Therefore, there is probably very limited access, if any, for specific antibodies to interact with their targets. Although not tested, it is unlikely that the local SIgA-mediated response to these proteins will be protective.

### **III-Characterization of the serotype-specific antigenic determinants of *S. flexneri* serotype2a lipopolysaccharide**

Antigenic determinants recognized by protective monoclonal antibodies were characterized in a competition ELISA using synthetic di-, tri-, tetra- and pentasaccharides obtained by circular permutation of the residues from the repetitive units of the O-SP from *S. flexneri* serotype2a (Figure 29), as well as longer fragments (octa- and deca-saccharides), as competitors for binding of the antibodies to the homologous LPS.

#### **A) Material and methods**

##### **1) Synthetic oligosaccharides representative of *S. flexneri* serotype2a**

##### **O-SP**

Oligosaccharides representative of fragments of the O-SP of *S. flexneri* 2a were synthesized by multistep chemical synthesis, as described in the preceding examples.

**Table A: Oligosaccharides\* representative of fragments of the O-SP of *S. flexneri* 2a**

Di-saccharide	Tri-saccharide	Tetra-saccharide	Penta-saccharide	Octa-saccharide	Deca-saccharide
AB	ABC				
BC	BCD				
DA	CDA				
(E)C	DAB				
(BE)C	B(E)C	AB(E)C	DAB(E)C		{DAB(E)C} <sub>2</sub>
	(E)CD	B(E)CD	B(E)CDA	B(E)CDAB(E)C	
	A(BE)C	(E)CDA	(E)CDAB		
			AB(E)CD		

The oligosaccharides were synthesized as methyl glycoside in order to mimic the glycosidic linkages present in the natural polysaccharide and prevent any ambiguity which may otherwise arise due to equilibrium mixtures of the  $\alpha$ - and  $\beta$ -anomers corresponding to the furanose and pyranose forms of the reducing residue.

5 The  $\beta$ EC and A( $\beta$ E)C compounds, which have a non natural EC glycosidic linkage, were synthesized in order to probe the influence of such linkage on Ab recognition. Since they were estimated to be the easiest chemically accessible targets, the octa- B(E)CDAB(E)C and deca-saccharide DAB(E)CDAB(E)C were chosen as the longer fragments in order to gain some knowledge on the length-dependent oligosaccharide-  
10 antibody recognition.

## 2) Monoclonal antibodies

The monoclonal antibodies specific for serotype 2a used in this study are the five IgG antibodies described in example X+1: F22-4, D15-1, E4-1, A-2, and C1-7. In addition, an IgG monoclonal antibody specific for serotype 5a (C20) was used as control.

## 3) Inhibition ELISA.

15 First of all, a standard curve was established for each antibody tested. Different concentrations of the antibody was incubated at 4°C overnight and then incubated on microtiter plates coated with purified *Shigella flexneri* LPS homologous to the strain used for the obtention of the antibody, at a concentration of 5 $\mu$ g/ml in carbonate  
20 buffer at pH 9.6, and previously incubated with PBS/BSA 1% for 30 min at 4°C. After washing with PBS-Tween 20 (0.05%), alkaline phosphatase-conjugated anti-mouse IgG was added at a dilution of 1:5000 (Sigma Chemical CO.) for 1 h at 37°C. After washing with PBS-Tween 20 (0,05%), the substrate was added (12 mg of p-nitrophenylphosphate in 1.2 ml of Tris, HCl buffer pH 8.8 and 10.8 ml of NaCl 5M). Once the color developed, the  
25 plate was read at 405 nm (Dinatech MR 4000 microplate reader). A standard curve OD= f(antibody concentration) was fitted to the quadratic equation  $Y = aX^2 + bX + c$  where Y is the OD and X is the antibody concentration. Correlation factor ( $r^2$ ) of 0.99 were routinely obtained.

30 Then, the amount of oligosaccharides giving 50% inhibition of IgG binding to LPS (IC<sub>50</sub>) was then determined as follows. IgG at a given concentration (chosen as the minimal concentration of antibody which gives the maximal OD on the standard curve) was incubated overnight at 4°C with various concentrations of each of the oligosaccharides to be tested, in PBS/BSA 1%. Measurement of unbound IgG was performed as described in the preceding example, using microtiter plates coated with  
35 purified LPS from *S. flexneri* 2a and the antibody concentration was deduced from the standard curve. Then, IC<sub>50</sub> was determined.

## 4) mIgG sequence analysis

Total RNA was extracted from hybridoma cells by RNAXel kit (EUROBIO). mRNA was converted into cDNA with a reverse transcriptase kit (INVITROGEN) and used as template for PCR amplification using Taq DNA polymerase (GIBCO, BRL) according the manufacturer's protocol. The amplification was performed with the primer of corresponding isotype (SEQ ID NO: 1 to 3; IgG1 : 5' GCA AGG CTT ACT AGT TGA AGA TTT GGG CTC AAC TTT CTT GTC GAC 3' ; IgG2a : 5' GTT CTG ACT AGT GGG CAC TCT GGG CTC 3' ; IgG3 : 5' GGG GGT ACT AGT CTT GGG TAT TCT AGG CTC 3'. The following eight heavy chain variable region (VH) primers were also used (SEQ ID NO: 4 to 11: 5' GAG GTG CAG CTC GAG GAG TCA GGA CC3' ; 5' GAG GTC CAG CTC GAG CAG TCT GGA CC 3' ; 5' CAG GTC CAA CTC GAG CAG CCT GGG GC 3' ; 5' GAG GTT CAG CTC GAG CAG TCT GGG GC 3' ; 5' GAG GTG AAG CTC GAG GAA TCT GGA GG 3' ; 5' GAG GTA AAG CTC GAG GAG TCT GGA GG 3' ; 5' GAA GTG CAG CTC GAG GAG TCT GGG GG 3' ; 5' GAG GTT CAG CTC GAG CAG TCT GGA GC 3'). Nucleic acid sequences were carried out by GENOME EXPRESS S.A. using PCR products. Sequence analysis was performed with software package from the Genetics Computer Group, Inc (Madison, WI), the Genbank (Los Alamos, NM) and EMBL (Heidelberg, Germany) databases. For the determination of the genes families, analysis of the nucleotide sequences was performed with the international ImMunoGeneTics database (<http://imgt.cines.fr>) (Lefranc, M.-P., 2003 *Nucleic Acids Res.*, 31, 307-310).

## **B) Results**

### **1) Antigenic determinants of *S. flexneri* serotype2a lipopolysaccharide**

The binding of the five available protective mIgGs to 25 synthetic mono- and oligosaccharides was evaluated in inhibition ELISA (Table B).

**Table B: Minimal sequence recognized by the mIgG**

Motif (*)	F22-4 IgG1 IC <sub>50</sub> (μmol/L)	D15-7 IgG1 IC <sub>50</sub> (μmol/L)	A2-1 IgG2a IC <sub>50</sub> (μmol/L)	E4-1 IgG2b IC <sub>50</sub> (μmol/L)	C1-7 IgG3 IC <sub>50</sub> (μmol/L)
<b>CD</b>	>1000	>1000	>1000	>1000	>1000
<b>EC</b>	>1000	>1000	>1000	>1000	>1000
<b>B(E)C</b>	>1000	>1000	>1000	>1000	>1000
<b>(E)CD</b>	<u>179</u>	>1000	>1000	>1000	>1000
<b>(E)CDA</b>	181	>1000	>1000	>1000	>1000
<b>(E)CDAB</b>	354	>1000	>1000	>1000	>1000
<b><u>B</u>(E)CD</b>	<u>5</u>	<u>198</u>	>1000	<u>87</u>	>1000
<b><u>B</u>(E)C<u>D</u><u>A</u></b>	2,5	240	<u>350</u>	75	<u>400</u>
<b>AB(E)C</b>	>1000	>1000	>1000	>1000	>1000
<b>DAB(E)C</b>	>1000	>1000	>1000	>1000	>1000
<b>AB(E)C<u>D</u></b>	21	490	378	287	734

(\*) Oligosaccharides are methyl glycosides derivatives

None of the mono- or disaccharides showed any binding when used at a concentration of 1 mM. Evaluation of trisaccharide recognition outlined the unique behaviour of mIgG F22-4, which was the only Ab showing measurable affinity for such short oligosaccharides. **ECD** was the only trisaccharide recognized by F22-4, pointing out the crucial contribution of both the branched glucosyl residue (**E**) and the *N*-acetylglucosaminyl residue (**D**) to Ab recognition. This was supported by the absence of recognition of **AB(E)C** or **DAB(E)C** by none of mIgG. Comparison of the recognition of the branched tetrasaccharide **B(E)CD** to that of the linear **ECD** indicated that rhamnose **B**, accounting for an improvement of the IC<sub>50</sub> by a factor of ~50, was also a key element in the Ab recognition. Indeed, **B(E)CD** was recognized by all the protective mIgG, except A2-1 and C1-7 for which the minimal sequences necessary for recognition were pentasaccharides **AB(E)CD** or **B(E)CDA**. Extension of **B(E)CD** at the reducing end, yielding the branched pentasaccharide **B(E)CDA**, did not result in any major improvement of Ab binding for the other mIgGs. The minor, if not absent, contribution of reducing **A** to binding was also apparent when comparing recognition of **ECD** and **ECDA** by F22-4. Further elongation at the reducing end, yielding **ECDAB** did not improve binding to F22-

4. Introduction of residue **A** at the non reducing end of **B(E)CD**, leading to **AB(E)CD**, has a somewhat controversial impact on Ab recognition with a positive effect in the case A2-1, and only a slight effect in the case of C 7-1, and even negative by a factor ~2 to ~4 when considering the other antibodies. Therefore, for the recognition of short oligosaccharides, two families of mIgGs were identified. The first one represented by F22-4 recognizing the **ECD** trisaccharide, and the second one, comprising the remaining four mIgGs, that recognized the same common **ECD** sequence flanked by the **B** residue at the non reducing end, added or not with **A** residue at the non reducing or reducing end.

This observation was confirmed when measuring the recognition of longer oligosaccharides (Table C).

**Table C: Antibody recognition is improved with longer oligosaccharides**

Anticorps	(*) <b>B(E)CDA</b> IC <sub>50</sub> (μmol/L)	<b>AB(E)CD</b> IC <sub>50</sub> (μmol/L)	<b>B(E)CDA B(E)C</b> IC <sub>50</sub> (μmol/L)	<b>DA B(E)CDA B(E)C</b> IC <sub>50</sub> (μmol/L)
<b>F22-4 (IgG1)</b>	2,5	21,6	0,22	5
<b>D15-7 (IgG1)</b>	240	490	60,8	11,9
<b>A2-1 (IgG2a)</b>	350	378	12,9	3
<b>E4-1 (IgG2b)</b>	75	287,7	12	4,4
<b>C1-7 (IgG3)</b>	400	734	242	19

(\*) All oligosaccharides are methylglycosides derivatives

Indeed, the decasaccharide was the highest affinity ligand for all antibodies except F 22-4. In the latter case, the octasaccharide was the best recognized sequence with an IC<sub>50</sub> of 0.22 μM, corresponding to an improvement by a factor ~10, when compared to pentasaccharide **B(E)CDA**. Further extension of the octasaccharide by addition of **DA** at the non reducing end resulted in a loss of recognition by a factor of ~20. Interestingly, the recognition of these two longer oligosaccharides by the other mIgGs differed from that of F22-4. D15-7 and E4-1 behaved similarly, with extension by **B(E)C** at the reducing end leading to the octasaccharide, and then by **DA** at the non reducing end, leading to the decasaccharide, both resulting in improving Ab binding by a factor of ~4. C1-7 behaved somewhat differently since contribution of **B(E)C** to binding appeared to be minor, whereas introduction of **DA**, resulted, as for the above cited mIgG, in an overall gain in binding of ~20. Finally, in the case of A2-1, addition of **B(E)C** to the reducing end of pentasaccharide **B(E)CDA** resulted in a gain in recognition by a factor ~25, and subsequent addition of **DA** at the non reducing end further contributed to binding improvement by a factor of ~4. To summarize, lengthening the oligosaccharide sequence improved the Ab recognition.

Thus, the data presented indicate the presence of an immunodominant epitope **(E)CD** of *S. flexneri* serotype 2a lipopolysaccharide, with flanking residues

contributing to the recognition depending on the monoclonal antibody. The sequences B(E)CDA and AB(E)CD are almost similarly recognized by all the monoclonal IgG antibodies. In addition, the recognition improvement observed with longer oligosaccharides indicate that multiple epitopes along the polysaccharide chain (17 repetitive units in average) but not one unique epitope at the extremity, are presented on the LPS.

## 2) Molecular characterization of the protective *S. flexneri* serotype 2a-specific mIgG

To analyse whether the differences observed in the recognition of oligosaccharides by the mIgGs reflect differences in the structure of these mAbs, their complementary-determining regions (CDRs) were sequenced (Table D and E).

**Table D: VH domain CDR sequences**

VH	CDR1 (SEQ ID NO : 12 to 15)	CDR2 (SEQ ID NO : 16 to 19)	CDR3 (SEQ ID NO : 20 to 23)	OLIGO- SACCHARIDE MOTIF
F22-4	NYWMS	EIRLKSDNYATYYAESVKG	PMDY	ECD
D15-7	YSSIH	WINTATGEPTYPPDDFKG	YDYAGFYW	B(E)CD
A2-1	DYSLH	WINTETGEPAYADDFKG	YRYDGAY	B(E)CDA
E4-1	DYSMH	WVNTQTGEPSYADDFKG	YRYDGAH	B(E)CD
C1-7				B(E)CDA

**Table E: VL domain CDR sequences**

VL	CDR1 (SEQ ID NO : 24 to 27)	CDR2 (SEQ ID NO : 28 to 31)	CDR3 (SEQ ID NO : 32 to 34)	OLIGO- SACCHARIDE MOTIF
F22-4	RSSKSLLHSDGITYLY	HLSNLAS	AHNVELPRT	ECD
D15-7	SASSSVGYIH	DTSKLAS	QQWSRNPLT	B(E)CD
A2-1	RATSSVGYIN	ATSNLAA	QQWSSDPFT	B(E)CDA
E4-1	RARSSVGYM	ATSNQAS	QQWSSDPFT	B(E)CD
C1-7				B(E)CDA

Only two VH and Vx gene families were expressed among the five studied mIgG (Table F).



**Table F: V gene usage**

mAb	isotype	VH	D	JH	VK	JK
A2-1	IgG2a	VGAM3-8	SP2	JH3	VK4/5	JK4
C1-7	IgG3					
D15-7	IgG1	VGAM3-8	SP2	JH3	VK4/5	JK5
E4-1	IgG2b	VGAM3-8	SP2	JH3	VK4/5	JK4
F22-4	IgG1	J606	not known	JH4	VK24/25	JK1

VH J606 (Brodeur et al., *Eur. J. Immunol.*, 1984,14, 922-930) and VK24/25 (Almagro et al., *Immunogenetics*, 1998, 47, 355-363) encoded F22-4 VH and V $\kappa$ , respectively. A2-1, D15-7 and E4-1 VH genes were members of the VGAM3-8 family (Winter et al., *Embo J.*, 1985, 4, 2861-2867) and their V $\kappa$  genes belonged to the VK4/5 family (Almagro et al., precited) . The joining segment of F22-4 heavy chain was encoded by JH4 (Sakano et al., *Nature*, 1980, 86, 676-683), while A2, D15-7 and E4-1 heavy chains shared the same diversity and joining segments, DSP2 (Gu et al., *Cell*, 1991, 65, 47-54) and JH3 (Sakano et al.,precited), respectively. The joining segment for the light chain is encoded by JK1 (Max et al., *J. Biol. Chem.*, 1981, 256, 5116-5120) for F22-4, JK4 for A2 and E4-1, and JK5 for D15-7. The four antibody CDRs except for CDRH3, fall into the canonical structure classes (Al-Lazikani et al., *J. Mol Biol*, 1997, 273, 927-948). For all mIgG, the CDRs L2, L3 and H1 were of the same classes, 1/7A, 1/9A and 1/10A, respectively (Martin et al., *J. Mol Biol.*, 1996, 263, 800-815). For F22-4, the canonical form of the loops L1 and H2 were of the classes 4/16A and 4/12A, while those of the three other antibodies fall into classes 1/10A for L1 and 2/10A for H2. The CDR-H3 of A2, D15-7 and E4-1 contained seven residues along with several aromatic ones, while the CDR-H3 of F22-4 was very short, only four amino-acids with a proline residue in the first position.

mIgG F22-4 binds to the O-SP in an unique mode, selecting the linear trisaccharide ECD as the minimal sequence necessary for recognition at a concentration below 1mM. The specificity of F22-4 suggests that the glucose residue (E) is probably involved in direct interactions with the Ab, while for the other mAbs, E may also constrain the conformation of another part of the oligosaccharide that interacts with the Ab. F22-4 uses a VHJ606/VK24/25 pair. The J606 family comprises VH genes encoding the immune response to  $\beta$ -(1,6)-galactan (Hartman et al., 1984, 3, 2023-2030). The CDRs H1, H2, L1 and L2 are quite similar in sequence and/or length to those of SYA/J6 (Table G), a mAb generated in response to immunization with *S. flexneri* Y.

**Table G: Comparison of the sequences of SYA/J6 (SEQ ID NO: 12, 35 to 39) and F22-4 (SEQ ID NO: 12, 16, 20, 24, 28 and 32) CDRs\***

	VH	H1	H2	H3			
		31 35	52abc	100a			
5	SYA/J6	NYWMS	EIRLKSNNYATHYA	ESVKG	GGAVGAMDY		
	F22-4	NYWMS	EIRLKSDNYATYYA	ESVKG	PM	DY	
	VH	L1	L2	L3			
		27abcde	30	50	56	89	97
10	SYA/J6	RSSQSL	LHSDGNTYLH	KVSNRFS	SQTTHVPT		
	F22-4	RSSKSL	LHSDGITYLY	HLSN	LAS	AHNVELPRT	

\* Kabat numbering

In contrast, the H3 loops, which are the major key of Ab diversity, are very different. In mAb SYA/J6, the CDR-H3 comprises nine amino-acids; its base which possesses three Gly residues, shows the torso-bulged structure (Morea et al., J. Biol. Chem, 1998, 263, 269-294) and this mAb is an example of a groove like site for binding an internal oligosaccharide epitope (Vyas et al., Biochemistry, 2002, 41, 13575-13586). In the case of F22-4, the H3 loop-four residues, which can only form a short hairpin, would allow a more open binding site, than can accommodate the linked glucose.

The improved F22-4 recognition of the tetrasaccharide B(E)CD outlines the key input on the branching site. However, as found in the case of pentasaccharide AB(E)CD and decasaccharide DAB(E)CDAB(E)C, further extension at the non reducing end of this key fragment had a negative impact on binding. These findings suggest that although the Ab combining site is most probably of the groove type, it is somewhat restricted on one side and unable to accommodate inappropriate extension.

The other mIgGs require B(E)CD as the minimal sequence recognized at a concentration below or close to 1mM (A2-1). These mAbs probably bind intrachain epitopes, as it is supported by the fact that the longer the oligosaccharide, the better the recognition. It is somewhat puzzling to note that although binding to the shorter oligosaccharides is slightly different, all the mIgGs fall into the same pattern of affinity when considering the decasaccharide. The most striking observation concerns A2-1, for which a 100 fold increase in binding was noted when comparing DAB(E)CDAB(E)C to B(E)CDA. It is noteworthy that these mIgGs use a VGAM3-8/VK24/25 pair, thus differing from F22-4. The VGAM3.8 multigene family was isolated from the DNA of mouse B-lymphocytes stimulated by LPS (Winter et al., Embo J., 1985, 4, 2861-22867).

Taken together, these results suggest that the particular behaviour of F22-4 in recognizing of the trisaccharide ECD, in comparison to the other mIgGs, could be related to particular molecular structure.

#### **IV. Preparation of TT conjugates**

##### **a) Material and methods**

*N*-( $\gamma$ -maleimidobutyryloxy) sulfosuccinimide ester (sulfo-GMBS) was purchased from Pierce. Tetanus toxoid (TT) (MW 150 kDa) (batch n°FA 045644), was purchased from Aventis Pasteur (Marcy l'Etoile, France), and stored at 4°C in a 39.4 mg.mL<sup>-1</sup> solution.

Dialyses were performed with Slide-A-Lyzer® Dialysis Cassettes (Pierce) and concentration by centrifugation using Vivaspin 15R centrifugal concentrators (Vivascience, Palaiseau, France), displaying a membrane cut-off of 10000 Da, at a centrifugal force of 4500  $\times$  g.

##### **i) pmLPS-TT conjugates**

###### *Preparation and derivatization of S. flexneri 2a pmLPS*

*S. flexneri* 2a LPS was treated with acetic acid to hydrolyse the lipid A-core linkage: LPS [10 mg in 1% (v/v) aqueous acetic acid (1 mL)], was heated at 100°C for 60 min. Precipitated lipid A was removed by low-speed centrifugation (350  $\times$  g for 15 min) at 4°C. The supernatant was extracted with equal volume of chloroform-ethanol (2:1). The reaction mixture was shaken vigorously and centrifuged at 10,000  $\times$  g for 60 min at 4°C. The aqueous phase was dialyzed against distilled water to remove ethanol and then freeze-dried to give *S. flexneri* 2a pmLPS (5.3 mg, 53%).

*S. flexneri* 2a pmLPS (2.2 mg, 0.13  $\mu$ mol) was dissolved in water (430  $\mu$ L) at an actual concentration of 5 mg.mL<sup>-1</sup>. The solution was brought to pH 11 with 2 N NaOH, and an equal weight of CNBr (4.0  $\mu$ L of a 5 M solution in CH<sub>3</sub>CN) was added. The pH was maintained at 11 with 2 N NaOH for 6 min at rt. An equal volume of adipic acid di-hydrazide (430  $\mu$ L of a 0.5 M solution in 0.5 M NaHCO<sub>3</sub>) was added, and the pH was adjusted to 8.5 with 0.5 M HCl. The reaction mixture was kept overnight at 6°C and dialyzed against 0.1 M potassium phosphate buffer at 4-6°C.

The extent of derivatization of the activated pmLPS was calculated as the ratio of adipic acid dihydrazide/polysaccharide (w/w) and found equal to 3.7% using trinitrobenzenesulfonic acid (TNBS), as titration reagent (Habeeb, A. F., *Anal. Biochem.*, 1966, 14, 328-336).

###### *Preparation and characterization of the conjugate*

The activated *S. flexneri* 2a pmLPS (1.8 mg), and the succinic anhydride treated TT (1.8 mg) were mixed. Solid 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) (5.3 mg), was then added to a final concentration of 0.1 M and the pH of the reaction mixture was maintained at 6 for 4 h at rt. The crude mixture was dialyzed against

PBS 1× (3 × 2 L) at 4-6°C and passed through a CL-6B Sepharose column (1 m × 160 mm) (Pharmacia Biotech), using 0.05 M PBS, pH 7.4 as eluent at a flow rate of 0.2 mL.min<sup>-1</sup>, with detection by measuring the optical density at 280 nm and the refractive index. The fractions containing the conjugates were pooled and concentrated. The conjugate was stored at 4°C in the presence of thimerosal (0.1 mg.mL<sup>-1</sup>) and assessed for its total carbohydrate and protein content.

## ii) Oligosaccharide-TT conjugates

### Derivatization of TT

In a representative example, to a solution of TT (12 mg, 304 µL, 0.08 µmole) diluted in 0.1 M PBS, pH 7.3 (296 µL), was added *N*-(γ-maleimidobutyryloxy) sulfosuccinimide ester (GMBS) (3 × 1.53 mg, 3 × 58 µL of an 30 mg.mL<sup>-1</sup> solution in CH<sub>3</sub>CN, 3 × 50 equiv), in three portions every 40 minutes. The pH of the reaction mixture was controlled (indicator paper) and maintained at 7-7.5 by addition of 0.5 M aq NaOH. Following an additional reaction period of 40 minutes, the crude reaction mixture was dialyzed against 3 × 2 L of 0.1 M potassium phosphate buffer, pH 6.0 at 4°C to eliminate excess reagent. About 45 maleimide groups were introduced on TT as indicated by SELDI-TOF MS analysis.

### Conjugation

Following dialysis, maleimide activated-TT in 0.1 M potassium phosphate buffer solution was divided into several portions which were further reacted with synthetic *S*-acetylthioacetylated-tri-, tetra- penta-, hexa-, deca- and pentadecasaccharides related to *S. flexneri* 2a O-SP in a 1:12 molar ratio, respectively. Reaction mixtures were buffered at a 0.5 M concentration by addition of 1 M potassium phosphate buffer, pH 6.0. Then, NH<sub>2</sub>OH, HCl (7.5 µL of a 2 M solution in 1 M potassium phosphate buffer, pH 6), was added to the different mixtures and the couplings were carried out for 2 h at rt. The conjugated products were dialyzed against 3 × 2 L of 0.05 M PBS, pH 7.4 at 4°C, and further purified by gel permeation chromatography on a sepharose CL-6B column (1 m × 160 mm) (Pharmacia Biotech), using 0.05 M PBS, pH 7.4 as eluent at a flow rate of 0.2 mL.min<sup>-1</sup>, with detection by measuring the optical density at 280 nm and the refractive index. The fractions containing the conjugates were pooled and concentrated. The conjugates were stored at 4°C in the presence of thimerosal (0.1 mg.mL<sup>-1</sup>) and assessed for their total carbohydrate and protein content.

In an attempt to maximize the loading of the protein, the derivatized-TT was reacted as described above but in a 1:56 molar ratio using the pentadecasaccharide related to *S. flexneri* 2a O-SP.

Hexose concentrations were measured by a colorimetric method based on the anthrone reaction, using pmLPS as a standard.

Protein concentrations were measured by the Lowry's spectrophotometric

method, using BSA as a standard and/o total acidic hydrolysis (6 N HCl at 110°C for 20 h), using norleucine as an internal standard.

Determination of hexoses with anthrone

*Reagents:* The reagents are as follows

5 Stock sulfuric acid. Add 750 mL of concentrated sulfuric acid to 250 mL of distilled water and cool the solution to 4°C.

Anthrone reagent. Dissolve 1.5 g of anthrone in 100 mL of ethyl acetate and cool the solution to 4°C.

10 Standard oligosaccharide solution: Prepare a solution at a concentration of 4 mg.mL<sup>-1</sup> in water. Prepare serial dilutions of 400 to 25 µMol of a tetra- or pentasaccharide [B(E)CD and AB(E)CD, respectively] standard solution in water. The tetra- and pentasaccharide standard solutions were used to dose the conjugates obtained using tri-, tetra-, penta- or hexa-, deca- and pentadecasaccharide, respectively.

*Procedure:*

15 Prepare serial dilutions of 400 to 25 µMol of the appropriate oligosaccharide standard solution in water (1 mL) in screw-threaded tubes. Prepare similarly a reagent blank containing 1 mL water and control reagents containing a known amount of pmLPS of *S. flexneri* 2a O-SP or glucose in 1 mL water. Prepare samples and make up to 1 mL if necessary by adding water. Cool all tubes in ice-water.

20 To each tube, add 5 mL of the concentrated H<sub>2</sub>SO<sub>4</sub> and 0.5 mL of the anthrone solutions. Heat the tubes at 100°C, caps unscrewed for 3 minutes and then caps screwed for 7 minutes. After exactly 10 minutes, return the tubes to an ice-bath and when cool measure the absorbance in a spectrophotometer (Seconam S.750I), at a wavelength of 625 nm. The quantity of carbohydrate in the unknown samples can be read off from the  
25 standard curve prepared with the standard solution samples and the blank.

b) Results

Characteristics of representative conjugates are listed in Table L.

TABLE L

Conjugate reference	Hapten	Isolated yield	carbohydrate /protein wt/wt (%)	Hapten/protein (mmol/mmol)
CGS0303-8-3	(E)CD	70%	5.4	12
CGS0303-8-4	B(E)CD	64%	7.4	13.3
CGS0303-8-5	AB(E)CD	80%	9.6	14.7
CIMG745	B(E)CD	66%	6.5	10.8
CIMG746	AB(E)CD	85%	6.5	10.9
CGS0703-56-10	[AB(E)CD] <sub>2</sub>	71%	16	13.5
CGS0703-56-15	[AB(E)CD] <sub>3</sub>	67%	43	24
CGS0104-113-4	B(E)CD	52%	12	15.8
CGS0104-113-5	AB(E)CD	51%	10	13
CGS0104-113-6	DAB(E)CD	72%	13	17
CGS0104-113-10	[AB(E)CD] <sub>2</sub>	62%	22	14
CGS0104-113-15	[AB(E)CD] <sub>3</sub>	68%	4	26
CGS0204-121	pmLPS	88%	41	3.6 <sup>a</sup>
CGS0703-51	pmLPS	74%	25	2.2 <sup>a</sup>

<sup>a</sup> Based on an estimated Mr of 17,000 kD for pmLPS (pmLPS stands for LPS detoxified by acid hydrolysis)

#### **V-Immunogenicity of the oligosaccharides-tetanus toxoid conjugates**

##### **A) Material and methods**

##### **1) immunization protocol**

Two immunization assays in the absence of adjuvant were performed with oligosaccharides conjugated to tetanus toxoid, prepared as described in preceding example.

In a first assay, groups of eight mice received four intramuscular injections at three weeks interval of B(E)CD, AB(E)CD, DAB(E)CD, [AB(E)CD]<sub>2</sub> or [AB(E)CD]<sub>3</sub> oligosaccharides conjugated to tetanus toxoid (10 µg oligosaccharide/mice/injection). Control mice received detoxified LPS from *S. flexneri* 2a conjugated to tetanus toxoid (10 µg polysaccharide /mice/injection) by multipoint attachment, as described by Taylor et al., Infect. Immun., 1993, 61, 3678-3687, or tetanus toxoid alone (140 µg/mice/injection), following the same immunization schedule. One month after the last injection, the mice received a last boost of conjugates, in the same conditions.

In a second assay, groups of seven mice received three intramuscular injections at three weeks interval of B(E)CD, DAB(E)CD, and groups of fourteen mice received three intramuscular injections at three weeks interval [AB(E)CD], [AB(E)CD]<sub>2</sub> or [AB(E)CD]<sub>3</sub> oligosaccharides conjugated to tetanus toxoid (10 µg oligosaccharide/mice/injection). Control mice received detoxified LPS from *S. flexneri* 2a conjugated to tetanus toxoid (10 µg polysaccharide /mice/injection) by multipoint attachment, as described by Robbins J.B. (J. Infect. Dis. 161: 821-832), or tetanus toxoid

alone (140 µg/mice/injection), following the same immunization schedule. Seven days after the last injection, the mice received a last boost of conjugates, in the same conditions.

## 2) Antibody response analysis

The anti-LPS 2a, anti-oligosaccharides and anti-tetanus toxoid (TT) antibody response was analysed by ELISA, seven days after the third immunization (before the boost), and seven days after the boost. Microtiter plates were coated with the corresponding antigen in carbonate buffer pH 9.6, at a concentration of 5µg/ml, for the LPS. Biotinylated oligosaccharide solutions were adjusted to equimolar concentrations based on the amount of ligand present in the respective glycoconjugate and incubated with PBS/BSA 1% for 30 min at 4°C. Bound antibodies were detected by using peroxidase conjugated anti-mouse immunoglobulins. After washing with PBS-Tween 20 (0.05%) alkaline phosphatase-conjugated anti-mouse IgG was added at a dilution of 1:500 (SIGMA) for 1 h at 37°C. After washing with PBS-Tween 20 (0,05%), the substrate was added (12 mg of p-nitrophenylphosphate in 1.2 ml of Tris, HCl buffer pH 8.8 and 10.8 mM of NaCl 5M). Once the color developed, the plate was read at 405 nm (Dinatech MR 400 microplate reader). Antibody titers were defined as the last dilution of the sample giving an OD at least twice that of the control.

## 3) Protection studies

The mice immunized i.m. were challenged i.n. with 10<sup>8</sup> virulent bacteria 8 days after the boost. Measurement of bacterial load was performed 24 h post-infection, as described in example II.

## B) Results

The anti-LPS 2a, anti-oligosaccharides and anti-tetanus toxoid (TT) antibody response was analysed by ELISA, seven days after the third immunization (before the boost), and seven days after the boost.

**Table H : Anti-LPS 2a antibody response induced by tetra-and hexasaccharides conjugates J7 after third immunization (1) and J7 after boost (2)**

	TETRA		HEXA		TT		LPS/TT	
	anti-LPS		anti-LPS		anti-LPS		anti-LPS	
Mouse n°	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
1	0	0	0	0	0	0	400	1600
2	0	0	0	0	0	0	0	400
3	0	0	0	0	0	0	0	800
4	0	0	0	0	0	0	0	200
5	0	0	0	0	0	0	0	200
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0



**Table I : Anti-LPS 2a antibody response induced by penta-, deca- and pentadecasaccharides conjugates J7 after third immunization (1) and J7 after boost**

**(2)**

	<b>PENTA</b>		<b>DECA</b>		<b>PENTADECA</b>	
	<b>Anti-LPS</b>		<b>Anti-LPS</b>		<b>Anti-LPS</b>	
<b>Mouse n°</b>	<b>(1)</b>	<b>(2)</b>	<b>(1)</b>	<b>(2)</b>	<b>(1)</b>	<b>(2)</b>
1	0	6400	3200	12800	6400	12800
2	0	3200	3200	25600	12800	51200
3	0	400	800	800	12800	51200
4	0	200	1600	3200	25600	25600
5	0	0	400	1600	25600	51200
6	0	0	25600	400	6400	12800
7	0	0	0	800	3200	3200
8	0	0	0	3200	12800	25600
9	0	0	0	6400	6400	25600
10	0	0	0	12800	12800	25600
11	0	0	0	3200	6400	25600
12	0	0	0	800	12800	25600
13	0	0	0	0	6400	12800
14	0	0	0	0	3200	12800
Percentage of responders	0%	28,50%	42,85%	85,70%	100%	100%
Mean of antibody titers		1/728	1/2500	1/5200	1/11000	1/26000

**Table J : Anti-oligosaccharide antibody response induced by tetra-and hexasaccharides conjugates J7 after third immunization (1) and J7 after boost (2)**

Mouse n°	TETRA		HEXA	
	(1)	(2)	(1)	(2)
1	51200	51200	200	800
2	51200	51200	200	6400
3	51200	51200	0	12800
4	12800	51200	0	0
5	0	51200	0	0
6	0	200	0	0
7	0	0	0	0
Mean of antibody titers	1/24000	1/36600	1/50	1/3000

5 **Table K : anti-oligosaccharide antibody response induced by penta-, deca- and pentadecasaccharides conjugates J7 after third immunization (1) and J7 after boost (2)**

Mouse n°	PENTA		DECA		PENTADECA	
	(1)	(2)	(1)	(2)	(1)	(2)
1	800	6400	200	800	3200	12800
2	1600	12800	100	25600	6400	51200
3	200	1600	100	6400	6400	51200
4	800	12800	3200	800	6400	51200
5	800	1600	0	400	6400	102400
6	0	12800	0	12800	1600	12800
7	0	25600	0	3200	6400	51200
8	0	6400	0	800	3200	51200
9	0	6400	0	1600	3200	51200
10	0	25600	0	6400	12800	51200
11	0	800	0	200	3200	51200
12	0	1600	0	0	400	51200
13	0	6400	0	0	3200	51200
14	0	25600	0	0	800	51200
Mean of antibody titers	1/300	1/10500	1/250	1/4500	1/4500	1/49000

No anti-LPS antibodies are observed in the mice immunized with the tetra- and hexasaccharides conjugates despite of an anti-oligosaccharide antibody response.

5 Low levels of anti-LPS antibodies are observed in the mice immunized with the detoxified LPS conjugate.

High levels of anti-LPS antibodies are observed in the mice immunized with the penta-, deca and penta decasaccharides conjugates. However, the antibody response is improved with the longer oligosaccharide (pentadecasaccharide); after the third immunization 100 % of the mice receiving the pentadecapeptides present anti-LPS  
10 antibodies, as compared with 85 % and 30 % only, for the mice receiving the decasaccharide and the pentasaccharide, respectively. Moreover, the anti-LPS antibody titers as well as the homogeneity of the antibody response is higher in the mice immunized with the pentadecasaccharide.

## 2) Protection studies

15 The ability of the antibodies induced by immunization with the oligosaccharides-TT conjugate to protect against *Shigella* infection was assayed by active protection studies in the mouse model of pulmonary infection.

Protection as assessed by a reduction of the bacteria load, was observed with the penta, deca and pentadecasaccharides conjugates whereas neither the tetra- and  
20 hexa saccharides conjugates, nor the detoxified LPS conjugate induced protection (figure 34).